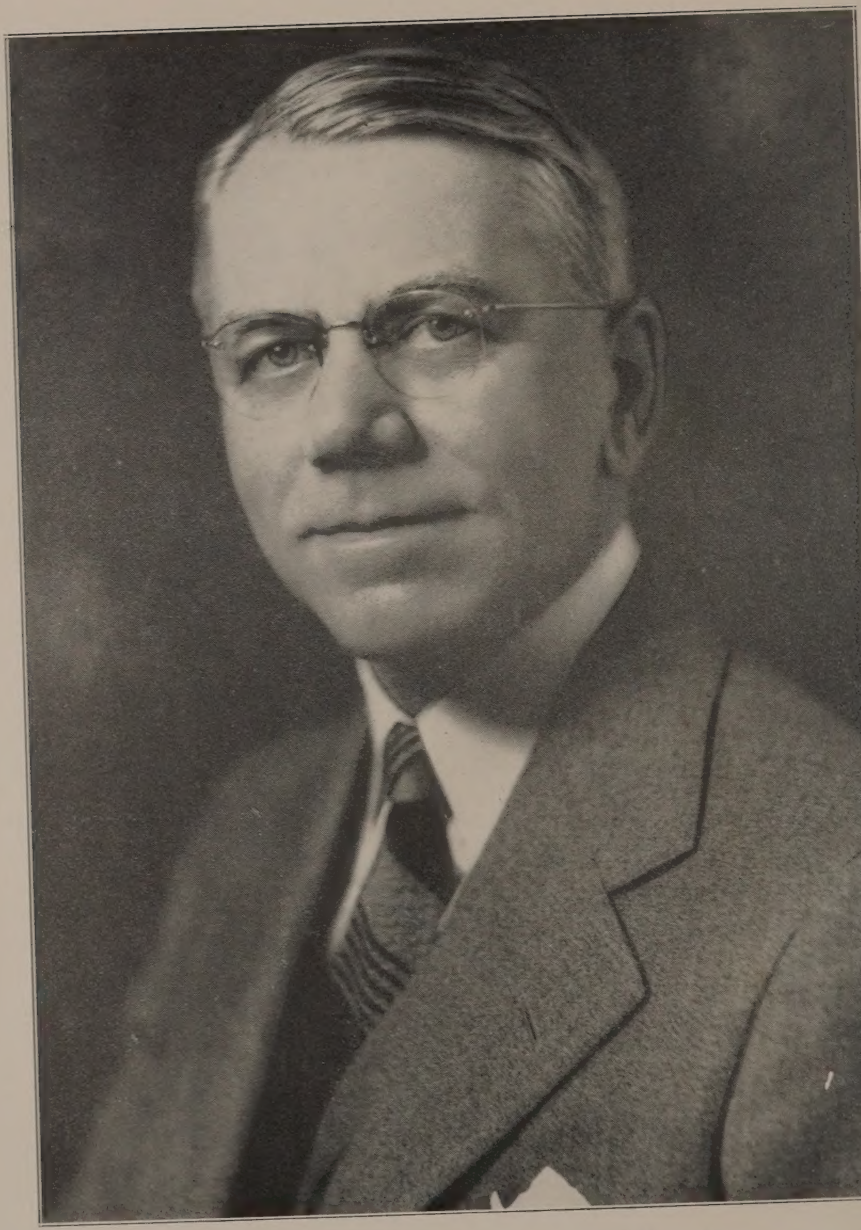

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THE RÔLE OF PARASITOLOGISTS IN WORLD WAR II*

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It is with a sense of real regret that this address is presented to the Society in print rather than by word of mouth. The New York members of the Society had looked forward with keen interest to welcoming a large attendance at what should have been one of its most noteworthy meetings. Your courteous gesture in electing to the presidency last year an amateur parasitologist from the medical profession has been accepted with appreciation of the fact that the Society recognizes, particularly in this time of national emergency, the importance of the animal parasites of man and the rôle they may play in winning or losing the world struggle in which we are involved.

The chief interest of the large majority of the parasitologists of the United States has been in the parasites of lower animals rather than in those of man. There are several reasons for this. The field is larger, the parasites are easier to obtain, they can be studied more easily in biological laboratories and do not require association with medical institutions. Furthermore, the animal parasites of man are less prevalent in most parts of the United States than they are in tropical countries. Our nation has not had extensive colonial possessions in the tropics and there has been less interest in intensive study and control of many of the human parasitic diseases than has existed in countries having extensive possessions or aspirations in the tropics.

Ever since the beginning of the present war in Europe in 1939, the Medical Departments of the Armed Forces of the United States have been concerned with the tropical and parasitic diseases which would face our troops if we were forced into the conflict. The point of view of those of us who had been closely associated with plans for the protection of our troops was limited by the focusing of attention on Europe, Africa and Latin America. With the Japanese attack on Pearl Harbor, however, the point of view of the whole nation was changed overnight and one dramatic element in that change was the sudden interest which the professional world and even the laity manifested in tropical diseases. Our horizon was suddenly extended to encompass the entire world and the diseases which would be encountered in the most remote and unprotected regions.

The use of total manpower means the use of each individual in the position where he can be best fitted, and for which he is best prepared, with the adaptation of his talents and experience to the winning of the war. The parasitologist has a place in the picture, and it is my purpose to sketch the picture so as to suggest the rôle that each may be able to play.

* Address of the Retiring President, American Society of Parasitologists, 1942.

Ever since the Spanish-American War the Medical Corps of the Army and Navy have been concerned with studying diseases which occurred among the personnel of those forces in tropical areas, and many of the studies arising from this interest have formed important contributions to the literature of human parasitology. The Veterinary Corps of the Army has also made valuable contributions to the study of diseases among animals used by land forces. The United States Public Health Service has steadily become more active in the study of diseases caused by animal parasites or transmitted by arthropod vectors. The United States Department of Agriculture, through a number of its divisions, has contributed in a most important manner to the health of domestic animals, and to the protection of man from parasites transmitted from lower animals. In addition to these federal agencies, state universities and private institutions have made important contributions to the control of animal parasites. All of these activities have built up a fund of knowledge and a corps of trained personnel which can act as a basis for the effective control of parasitic diseases among men and animals during the present conflict.

When the Surgeon General of the Army requested the National Research Council in the spring of 1940 to establish advisory committees to the Armed Forces in various fields of medicine, one of the first groups formed was the Subcommittee on Tropical Diseases. This Subcommittee of the Committee on Medicine accepted as its first responsibility the preparation of a series of directives for the diagnosis, treatment and prevention of certain tropical diseases. These were first issued as Circular Letter No. 56 of the Surgeon General's Office in June, 1941 (1) and were published as a section of the War Department Technical Manual 8–210 "Guides to Therapy for Medical Officers" in March, 1942. They were revised and a number of new ones added during the summer of 1942, and will be reissued in the near future. The Naval Medical School used these directives as the basis for a bulletin entitled "Notes on Tropical and Exotic Diseases of Naval Importance," the second edition of which was published in November, 1942.

The second activity of the Subcommittee on Tropical Diseases was the encouragement and supervision of research requested by the Armed Forces. Funds provided by the federal government made it possible to support such research through the Office of Scientific Research and Development, and numerous studies have been in progress during the past year or more on malaria, amoebiasis, insecticides and insect repellents, as well as on other tropical diseases outside the field of parasitology. Four members of the Society have been members of the Subcommittee on Tropical Diseases and many more have participated in the research sponsored by the Subcommittee.

The third interest of the Subcommittee on Tropical Diseases has been the encouragement of instruction in tropical diseases to medical officers and medical students. The Army and Naval Medical Schools had always stressed parasitology and tropical medicine in their post-graduate courses for medical officers, but these courses have recently been tremendously strengthened and expanded. In the summer of 1941, on the recommendation of the Subcommittee, the Army Medical School established a special two-months course for medical officers, with a faculty composed of authorities both within and outside of the government services. At first this course could accommodate only thirty students and the assignment of even that number was difficult to obtain. Recently, however, the Secretary of War issued an

order that every unit of troops going to the tropics should be supplied with a medical officer trained in Tropical Medicine. This has led to the expansion of the course to accommodate two hundred students, and in addition the Department of Tropical Medicine at Tulane University has been requested to give a similar course every two months to an additional thirty medical officers.

Other forms of specialized training which the Armed Forces are providing in the field of tropical medicine and parasitology include a short period of field instruction in malaria at the Tennessee Valley Authority and courses in clinical microscopy for medical officers and medical technicians at the army and naval medical schools and at a number of universities throughout the country. Members of the American Society of Parasitologists are participating in practically all of these courses of instruction to personnel in the Armed Forces.

Tropical medicine and parasitology have never held prominent place in the curriculum of most medical schools in the United States. The deplorable lack of instruction in these fields has long been recognized by a few individuals. At the meeting of the Association of American Medical Colleges in October 1941 a committee was appointed to make a study of the teaching of tropical medicine in medical schools. This committee published its preliminary report in the Journal of the Association in March 1942 (2), and presented its final report at the meeting of the Association in October (3). This report showed that only a small minority of the schools were giving adequate instruction in either tropical medicine or parasitology and that many of them lacked instructors qualified to teach these subjects.

Meanwhile, the effect of Pearl Harbor had been felt in the medical schools and a great interest developed in improving instruction in these fields. At the suggestion of Colonel James S. Simmons, Chief of the Division of Preventive Medicine of the Office of the Surgeon General of the U. S. Army, the John and Mary R. Markle Foundation made an appropriation to the National Research Council to develop a program of traveling lecturers on tropical medicine. The Markle Foundation has also made an appropriation to the Association of American Medical Colleges to enable instructors from the United States and Canadian medical schools to attend the intensive courses in tropical medicine at the Army Medical School and Tulane University. Each medical school was offered the opportunity of sending a clinical instructor to the Army Medical School and a pre-clinical instructor to Tulane. It was necessary to limit the enrollment in each of these courses to thirty students for the courses beginning January 4th, 1943. Both courses have been fully enrolled and a number of applicants had to be deferred. It is hoped that they can be given an opportunity to attend these courses at a later date. A number of members of the American Society of Parasitologists are included among the attendants at these courses.

Intramural courses in tropical medicine or parasitology anywhere in this country leave much to be desired in providing practical experience with diseases as they occur in the tropics. The armed forces have recognized this but their medical officers will have to get their practical experience in their assignments in the tropics. There is a probability, however, that instructors from medical schools who have taken the intensive courses in tropical medicine described above, may have an opportunity for a brief period of practical experience in the American tropics during the coming year. Colonel Simmons of the Army Medical Corps and General George C. Dunham,

Director of the Division of Health and Sanitation of the Office of the Coordinator of Inter-American Affairs, are cooperating in the development of such opportunities and Mr. Archie S. Woods of the Markle Foundation has expressed active interest in it.

Several medical schools have already strengthened their teaching staffs by the addition of a parasitologist. Some of these have been transferred from academic schools in the same institution while others have been obtained elsewhere. The loss of medical personnel from the staffs of medical schools may make funds available for the employment of a qualified parasitologist. Some medical school authorities still need stimulation to an appreciation of the importance of parasitic diseases in the medical school curriculum. Other schools may require additional funds for an instructor in this field. Parasitologists who are connected with such institutions may be able to assist in solving some of the problems of personnel.

One other aid to the teaching of tropical and parasitic diseases which has received attention is the provision of adequate teaching material. The Army Medical School has established a Distributing Center for parasitological specimens and other material which may be of value. The needs of the medical schools for teaching specimens were ascertained several months ago. The Army Medical School has circulated directions for obtaining material locally, and much material which is difficult to obtain locally will be furnished on request. The Office of the Surgeon General of the Army also plans to make available to medical schools the data which it has been accumulating on the distribution of diseases in tropical countries. Maps showing the world distribution of the important tropical diseases are also being prepared for distribution. The American Medical Association is cooperating in the editing of this material and the Markle Foundation is assisting financially through the National Research Council. This tremendous surge of interest and activity in preparing for the protection of our troops during the present war emphasizes the important rôle which parasitologists are taking and will take in the program from the educational and research points of view.

The opportunities for parasitologists to enter military service in their professional capacities depends mainly upon their individual qualifications. Only the largest military hospitals and laboratories require for routine operation officers who have qualifications limited exclusively to parasitology. Smaller laboratories are under the supervision of medical officers, most of whom have had special training in parasitology; and most of the enlisted personnel have also had a certain amount of training in the diagnosis of parasitic infections. The Army Sanitary Corps provides for non-medical commissioned officers, but bacteriologists are preferred to parasitologists because the routine work of laboratories lies more along bacteriological lines. An individual who has qualifications in both fields is likely to be especially favored in applying for a commission. The Navy has Hospital Volunteer Specialist Commissions in its Hospital Corps somewhat comparable to the Sanitary Corps of the Army.

There are certain assignments for teaching, research or control activities in the Army and Navy, for which experience in certain fields of parasitology are required. There may be greater opportunity for such assignments as the war goes on. Where special investigations have to be carried out or special control measures instituted, personnel with expert training will be required. Malaria is one of these fields, and although malaria units would require medical malariologists, sanitary engineers and

entomologists, there might be a need for protozoologists among the commissioned or enlisted personnel. The same might be true in other fields where protozoan or helminthic diseases become important enough to require special investigations or control measures.

At the request of the Office of the Surgeon General of the Army, Dr. Norman R. Stoll prepared a list of parasitologists who were known to be already in military or other government service, of others who were teaching in medical schools, and of still others who were available and apparently qualified for military service. More recently Dr. Stoll has supplemented this list by one which gives more details as to the experience of some of these persons in human parasitology. These lists should be of great assistance in the selection of parasitologists for military service and there is every indication that opportunities for active service will multiply as the war continues.

I have spoken of the rôle of parasitologists in active military service and in teaching during the present war. There is also a wide field open in research, on the control both of human parasitic diseases and of those of animals and plants. Not only is it necessary to keep our military and civilian population in the best of health, but it is necessary to furnish them with the best of animal and vegetable foods at the least possible cost. Domestic animals must be raised with a minimum of loss and with the maximum of food value. Crops must be improved, saved from the hazards of predatory insects, and preserved for safe delivery after storage and shipping to distant regions. In research laboratories, both institutional and commercial, parasitologists will undoubtedly find increasing opportunities to contribute to the war effort.

It is impossible to cover the field of opportunity in parasitological research, but I should like to mention a few of the unsolved problems in human parasitology. There are many unsolved problems in malaria. We do not yet know what becomes of the sporozoite between its introduction into man by the mosquito and the appearance of the first merozoites in the red blood cells. We have no drug which will attack the parasite in this most important stage when malaria might be prevented in the human host. We don't yet know the cause of the relapse in malaria, whether it is due to the smoldering of the asexual cycle in the spleen pulp where drugs will not reach it in sufficient concentration, or whether it is due to an exo-erythrocytic cycle in the reticulo-endothelial cells of the body. We do not know whether drugs attack the malaria parasite itself within the red cells or in the blood plasma, or whether they have an indirect action through cellular metabolism. No one has ever cultivated the malaria plasmodia outside the host.

The prevention of malaria by the control of mosquitoes still offers great opportunities for research. There is much to be learned about the methods of killing mosquito larvae. Mosquito repellents have only recently been studied in a systematic way, and the same is true of the methods employed in the killing of adult mosquitoes by sprays. All the problems of malaria control in hyperendemic areas will require a tremendous amount of study both during and after the present conflict.

Amoebiasis also presents some unsolved problems. We do not know how effectively sewage treatment prevents cysts from surviving in sludge or effluent. There is evidence that the usual chlorination of drinking water will not kill the cysts. The relative importance of different modes of transmission has not been completely

studied. The relationship of nutrition and bacterial infection to the invasion of the wall of the intestine is not sufficiently understood. *Endamoeba histolytica* has never yet been cultivated free from bacteria, and this has limited the possibilities of studying its action on tissues, its antigenic properties, and its growth requirements. In the treatment of amoebiasis there is room for improvement, particularly the discovery of a drug to replace emetine for parenteral therapy in order to avoid the toxic effects of that drug.

Trypanosomiasis also presents unsolved problems. In the African infections more effective drugs are needed particularly against the Rhodesian strain. There would be a great advantage also in having drugs which could be administered by mouth, both for the treatment and prophylaxis of the infection. Satisfactory methods of controlling tsetse flies are also needed, particularly in view of the fact that airplanes might easily carry these vectors to South America or India, and establish both vector and disease in new areas.

Trypanosoma cruzi is also unconquered with respect to treatment, nor do we know its exact limits of distribution and particularly whether endemic cases occur in the United States.

Leishmaniasis presents a number of unsolved problems. Only recently has convincing evidence been presented of its actual transmission to man by *Phlebotomus,* but we still do not know what factors determine the incubation period or whether the disease will develop at all in certain infected individuals. Better drugs are needed in treatment, especially for the cases in the Sudan which are resistant to antimony preparations. Again we need drugs which can be administered by mouth. The occurrence of visceral leishmaniasis in Brazil is not yet fully understood, particularly in its possible relationship to the cutaneous form of the disease. The relationship of canine to human infections and the importance of the dog as a reservoir host needs further study.

In helminthic infections the field of treatment still presents many opportunities particularly in connection with *Trichinella* and the filarial worms. Schistosomiasis is also a field for further research, including the possibility of its becoming endemic in the United States, the prevention of transmission in the extramammalian cycle, and the discovery of better drugs particularly for treatment by mouth instead of by vein.

These are illustrations of the fields of research which are open in human parasitology during the present war. The real problems in all fields are related to the prevention of human and animal diseases and the promotion of vigorous health. We should not be concerned so much with taxonomy and the morphological aspects of parasitology as with the action of harmful parasites and with methods of preventing their depredations. These are more difficult fields than those concerned with the anatomical structure of parasites, but they offer more promise of helpful results.

The second World War is only the beginning of the struggle which is before us. Unless we win the peace, we shall lose the war. We must not think that we can go back to our former fields of limited activity when the conflict is over. Our program now must be a preparation for world leadership in the future. Not only will the parasitologist be more important in this country after the return of troops with infections acquired in all parts of the world, but there will also be many opportunities for parasitologists to participate in the study and control of diseases throughout the

world. Military occupation followed by civilian guidance in improving health and economic welfare will create posts for research and campaigns of prevention. The personnel for much of this work will have to be trained in this country. Medical schools will appreciate the importance of presenting a world point of view to their students. Postgraduate instruction in tropical medicine and public health will be greatly increased. Philanthropic agencies will offer opportunities for the exchange of highly trained personnel between countries. Commercial interests will appreciate the importance of maintaining health among native employees in order to conserve their investments as well as to benefit the populations with whom they work.

This picture which I have tried to draw of the part which members of this Society are playing and will play in the second World War, should be a source of satisfaction as well as encouragement. There is no reason why the talents of each one should not be used to the fullest extent. Service at home in teaching or research offers just as valuable opportunities as service on the firing line. If any one feels that his talents are not being used to the fullest advantage, let him look about and see whether he can broaden his field of interest or fit himself better into the great mosaic of cooperative effort. Out of this war, with its destruction of peacetime complacency, postponement of meetings and disruption of academic curricula, should come a new era of scientific advancement which will be of benefit to all mankind.

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A DESCRIPTION OF *PHYLLODISTOMUM ETHEOSTOMAE* FISCHTHAL, 1942 (TREMATODA: GORGODERIDAE), FROM PERCID FISHES*

Jacob H. Fischthal

During the examination of the urinary bladder of a northern greenside darter, *Etheostoma blennioides blennioides* Rafinesque, collected on March 23, 1941, from the Huron River in Ann Arbor, Michigan, two immature and one mature specimens of a trematode belonging to the genus *Phyllodistomum* Braun, 1899, were found by Mr. Robert E. Kuntz of this laboratory. This material was kindly given to the author who determined it to be a new species. During the summer of 1941, the author found two of 19 northern greenside darters from the Saline River in Saline, Michigan, infected with one and two adult worms, respectively. Further investigations revealed that one of 21 blackside darters, *Hadropterus maculatus* (Girard), and one of 23 northern logperch, *Percina caprodes semifasciata* (De Kay), collected from Honey Creek near Ann Arbor, had one adult worm each in their

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* Contribution from the Department of Zoology, University of Michigan.

urinary bladders. The following description is based upon the study of living specimens, and of whole mounts of specimens fixed in hot 10 per cent formalin, stained in Mayer's paracarmine and mounted in clarite X (Nevillite no. 1).

A preliminary description of this species has appeared in an abstract by Fischthal (1942).

Phyllodistomum etheostomae Fischthal, 1942
(Figs. 1–2)

Specific diagnosis: Phyllodistomum. Body small, narrowly spatulate. Lateral margins of posterior portion of body thrown into two pairs of folds or flutes giving a wavy or undulate appearance. Posterior end of body slightly notched, turning ventrad. Cuticula spineless, bearing sensory papillae on suckers and on surfaces and margins of body. Oral sucker subterminal, larger than acetabulum; sucker ratios: lengths of oral sucker to acetabulum as 1 : 0.85; widths of same organs as 1 : 0.74. Mouth subterminal, ventral; esophagus weakly muscular, slender; intestinal crura narrow, ending at posterior fourth of body, lined with conspicuous layer of cells.

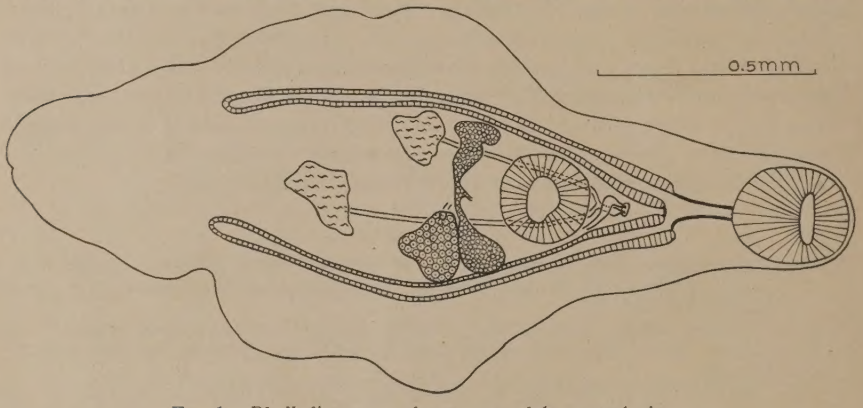

FIG. 1. *Phyllodistomum etheostomae*, adult, ventral view.

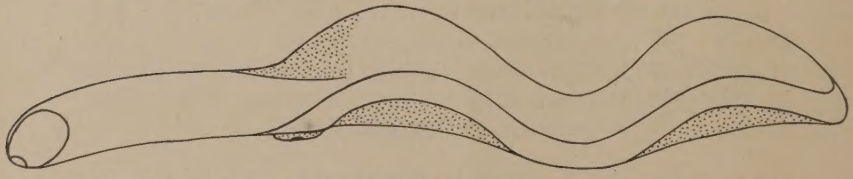

FIG. 2. Lateral view of *Phyllodistomum etheostomae* showing two pairs of marginal folds.

Vitellaria ovoid, smooth or slightly lobate, near and postero-lateral to acetabulum. Vitelline ducts joining dorso-mesially; common vitelline duct short, slightly swollen.

Ovary compact, equal to either testis or slightly larger, slightly and irregularly lobate, usually in contact with vitellarium, amphitypic. Oviduct short, arising from dorsum of ovary. Uterus extensive, coils intra- and extracecal, extending to margins of body posterior to acetabulum; uterus ascending dorsal to acetabulum, constricting to weakly muscular metraterm; genital atrium small. Eggs numerous; older intrauterine eggs containing fully developed miracidia. Measurements of 10 older embryonated intrauterine eggs: 0.031 (0.029–0.032) × 0.022 (0.021–0.024) mm. Testes oblique, well separated, irregularly lobate, indentations deeper and lobes more numerous than in ovary. Anterior testis not in contact with vitellarium and opposite or slightly oblique to ovary. Posterior testis behind but never in contact with ovary. Vasa efferentia arising from dorsum of testes, passing forward dorsal to acetabulum to their junction slightly anterior to acetabulum; vas deferens short; seminal vesicle small; ejaculatory duct short. Genital pore mid-ventral, approximately half-way between intestinal bifurcation and acetabulum. Excretory pore postero-dorsal; bladder a slender, median tube extending anteriorly to level of ovary where right and left primary collecting ducts enter.

Mean measurements in millimeters (with minima and maxima within parentheses) of five adult worms mounted in toto: Body, length 1.766 (1.475–1.995), width at intestinal bifurcation 0.288 (0.248–0.330), at anterior testis 0.738 (0.540–0.915); oral sucker, length 0.258 (0.195–0.300), width 0.228 (0.195–0.255); acetabulum, 0.188 (0.155–0.218) × 0.194 (0.180–0.225); esophagus, length 0.173 (0.160–0.180); left vitellarium, 0.144 (0.136–0.152) × 0.081 (0.056–0.099); right vitellarium, 0.130 (0.101–0.149) × 0.083 (0.067–0.136); ovary, 0.146 (0.117–0.173) × 0.139 (0.110–0.165); anterior testis, 0.148 (0.120–0.220) × 0.122 (0.101–0.147); posterior testis, 0.178 (0.140–0.225) × 0.129 (0.106–0.165).

Hosts and localities: Etheostoma blennioides blennioides Rafinesque from Huron and Saline Rivers; *Hadropterus maculatus* (Girard) and *Percina caprodes semifasciata* (De Kay) from Honey Creek; all in Washtenaw County, Michigan.

Habitat: Urinary bladder.

Type: U. S. Nat. Mus. Helm. Coll. No. 36820 (from *Etheostoma b. blennioides,* Saline River); paratypes in author's collection.

Of eight *P. etheostomae* examined, five had the ovary on the left and three on the right indicating that amphitypy occurs frequently in this species.

In possessing an oral sucker larger than its acetabulum *etheostomae* differs from all known species of *Phyllodistomum* with the exceptions of *brevicecum* Steen (1938), *caudatum* Steelman (1938), *mogurndae* Yamaguti (1934), *pearsei* Holl (1929) and *unicum* Odhner (1902). This species differs from *brevicecum* in possessing two pairs of marginal folds instead of a single pair, and from the other four which have no marginal folds. Of the six species compared, *etheostomae* and *mogurndae* have the posterior end of the body notched; *etheostomae* lacks the caudal projection characterizing *caudatum.* The present species is distinguished also in having its intestinal crura end at the posterior fourth of the body instead of at the posterior testis as in *brevicecum,* or near the posterior end of the body as in the other four species. It differs further from *caudatum* and *unicum* in having its uterus extensively developed extracecally as well as intracecally. It is distinguished further from *pearsei* in having sucker ratios of 1 : 0.85 × 1 : 0.74 instead of 1 : 0.59, and its testes are approximately twice as long as in *pearsei,* although their widths are similar. In having its body and eggs significantly smaller *etheostomae* is differentiated further from *mogurndae* and *unicum.*

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THE LIFE CYCLE OF FOUR INTESTINAL COCCIDIA OF THE DOMESTIC RABBIT

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Although infections with coccidia have long been recognized among domestic and game animals, considerable confusion still exists concerning classification of various species of coccidia, their life cycles and methods of control. Coccidia are of great economic importance to animal industry. Zoologists, pathologists and investigators in related fields have contributed information toward the control of these parasitic protozoa and one of the factors which aids in an intelligent interpretation of this problem is derived from a study of life cycles. While the control of coccidiosis is not entirely dependent upon a definite knowledge of the life cycles, such information does add appreciably, and in turn aids in instituting prophylactic measures.

In the domestic rabbit, *Oryctolagus* (Hobbs, 1931), five species of *Eimeria* occur. Four of these multiply in the epithelial cells of the small intestine and one in the epithelium of the bile passages. Early workers in this field failed to differentiate all four of these species because they often dealt with mixed infections. Kessel and Jankiewicz (1931) clarified the situation by differentiating all four on the basis of the morphological structure of their oöcysts. Accepting this differentiation it has been possible to segregate the four species, and establish pure infections of each in coccidia-free rabbits, thus enabling one to determine their endogenous life cycles. The species which is parasitic in the bile ducts was first noticed by Leeuwenhoek in 1674. He described bodies in the bile of rabbits which Dobell (1922) believes were the oöcysts of *Eimeria stiedae*. Hake (1839), according to Wenyon, first described the pathology of coccidiosis in the liver and duodenum of the rabbits, although he believed the oöcysts to be "pus globules" associated with carcinoma of the liver. No attempt was made, however, by the first investigator to differentiate between the species inhabiting the liver and intestine.

It was not until 1879 that Leuckart proposed the name *Coccidium perforans* for the coccidia he found to inhabit the intestine, and it is assumed that he described, for the most part, the species now known as *Eimeria perforans*.

Remak (1845) first noted oöcysts in the intestinal mucosa and Kauffmann (1847), according to Wenyon, observed that the oöcysts segmented into four separate sporocysts if kept in water. Stieda (1865) corroborated this observation and noticed in addition that within each sporocyst two elongated structures developed. These are now known as sporozoites. Balbiani (1884) proved that the sporocysts produce two sporozoites and a small residual body. It was Lindemann (1865), however, who recognized the parasitic nature of these cysts and called them *Monocystis stiedae*. In 1875 Schneider established the genus *Eimeria* for the coccidium of the mouse, and Leuckart's genus, *Coccidium*, is now considered a synonym for *Eimeria*.

L. Pfeiffer (1890a, 1891) and R. Pfeiffer (1892), according to Wenyon, were the first to indicate that an endogenous multiplication occurred in the rabbit's in-

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testine from which oöcysts developed. Simond (1897) fed oöcysts to young rabbits, reared from birth on sterile milk, and demonstrated the intermediate stages of schizogony, as well as newly formed oöcysts like the original ones fed.

Waworuntu (1924) described certain developmental stages of *E. perforans* and also a few for *E. stiedae* while Perard (1924a) also gives a few stages of development of *E. perforans* and *E. stiedae*. In 1924a, Perard reported a new intestinal species which in 1925b he named *Eimeria magna*. Prior to his description, other workers had considered this to be a larger form of *E. perforans* or had confused it with *E. stiedae*.

Waworuntu (1924) failed to differentiate between these three species and produced intestinal coccidiosis with what he thought to be *E. stiedae*. In reality he was working with *E. magna* as is shown in Plate II, Fig. 9 of his publication. Wenyon (1926) and Krijgsman (1926) also confused *E. magna* and *E. stiedae*.

Waworuntu (1924) reported a 3rd type of oöcyst, intermediate in size between *E. stiedae* and *E. magna*, which is really smaller than *E. magna*. This new type also possessed morphological differences and was named *E. media* by Kessel and Jankiewicz (1931). These workers named an additional species found in the intestine, *E. irresidua*, the oöcyst of which is easily confused with *E. stiedae*.

It is the purpose of the work here reported to determine in detail the endogenous phases of the life cycle of these four species and to correlate them with the exogenous phases previously described.

MATERIALS AND METHODS

The oöcysts of each of these four species of coccidia are easily differentiated by their characteristic appearance. Since as many as three species have been found to occur at one time in a naturally infected rabbit, the first problem was to isolate pure lines. This was accomplished in a variety of ways after the length of the life cycle for each species had been determined.

E. magna was the first one to be obtained free from the other species. This was accomplished by feeding to young coccidia-free rabbits, sporulated oöcysts of *E. magna* with which were found a few *E. perforans*. Pellets containing oöcysts were collected from these animals, after seven days, and allowed to sporulate for a period of from 4 to 5 days in shallow dishes to which was added sufficient 2% potassium dichromate solution to keep the feces covered. This procedure was repeated three times, and on each occasion the newly collected oöcysts were used to establish the new infection. After the 3rd successive feeding of the sporulated material, a pure strain of *E. magna* was obtained.

From a rabbit which was infected with both *E. magna* and *E. irresidua*, in which *E. irresidua* predominated, sections of the intestine were taken at intervals of three inches and examined for oöcysts by microscopic examination of the mucosal scrapings. Oöcysts of *E. irresidua* only were found to occur in the first six inches of the intestine, whereas in the lower regions they were found to occur together. These oöcysts of *E. irresidua* were allowed to sporulate and upon continued infection gave a pure line.

E. magna and *E. media* often occur together in infected rabbits. The former has a developmental cycle of seven days, while *E. media* requires but six days. By feeding a mixture of these species and by collecting pellets on the 6th day of infection, one is able to obtain oöcysts of *E. media* only. By removing all food on the 4th day

after administering the infective dose and then returning it between the 5th and 6th days, showers of oöcysts of *E. media* will be discharged.

E. perforans completes its life cycle in five days. By following the procedure as outlined above for *E. media*, one is able to obtain a pure strain of this small species.

After the desired species were obtained in pure form, they were routinely passed through two successive rabbits as a check against contamination. These oöcysts from pure species were collected either from cecal contents or the pellets, sporulated and fed to the coccidia-free animals used for the life cycle studies.

Before feeding sporulated oöcysts, contained in fecal material saturated with potassium dichromate, one must remove this chemical. This is easily accomplished by filling centrifuge tubes one-fourth to one-third full with feces, adding water, stirring, and centrifuging. All oöcysts and debris collect in the bottom and the supernatant fluid can be poured off. By adding a small amount of water to the sediment, the debris is easily removed from the centrifuge tube.

It was found that the slow passing of air or oxygen through the dichromate mixture of the feces insured the sporulation of a maximum number of oöcysts in the shortest possible time.

All rabbits used in these experiments were raised in separate isolation rooms. Pregnant does were separated from one another by placing them in sterilized wire cages which for added precautionary measures were changed every 48 hours. Food containers were also sterilized and changed with each exchange of cages.

After the young were a week old their nesting boxes were also changed every two days. The young were taken from their mothers, when 21 days old, and placed in small, sterilized, isolated cages in a separate room. These animals were checked every day for a week before infection and every day thereafter until the animals were sacrificed.

Infection was effected, in most cases, by injecting oöcysts directly into the stomach by means of a Luer syringe to which was attached a piece of small rubber tubing. Animals which were to be sacrificed during intervals of the first three days, were given a larger number of oöcysts than those infected for longer periods. This insured the presence of many more stages in the tissues for study.

An animal from each feeding experiment was sacrificed each 12 hours for the first three days and thereafter every 24 hours up to the 12th day. Tissues for histological studies were taken from 4 to 6 different regions of the intestine.

All tissues were fixed in Bouin's fluid, dehydrated, embedded in paraffin, and sectioned. After several stains were tried, it was decided that iron haematoxylin was superior to others. This was also employed for smears which were made from many regions of the intestine at the same time the tissues were taken.

All drawings were made in ink with the aid of a camera lucida, from stages found either in the tissues or on smears. The drawings were made to an original magnification of × 1700 except Figs. 31, 68, 91 and 117, which were × 2700.

EXPERIMENTAL RESULTS

Eimeria irresidua

The development of the intestinal parasite, *Eimeria irresidua*, Kessel and Jankiewicz (1931), occurs in the epithelium of the villi, from the duodenum to the lower ileum. The heaviest infection found was located in the first 18 inches of the gut

before the liberation of the 1st generation of merozoites. After these are freed, 2nd generation schizonts may develop at any place within the entire length of the gut.

Since this study deals with the endogenous cycle of this parasite, a suitable point at which to begin the description is immediately after excystment of the sporozoites in the intestine.

Rabbits killed after an infection of six hours show sporozoites (Figs. 1–3) free in the lumen and young schizonts developing in the epithelium. Sporozoites appear to remain in an infective condition in the intestine for at least three days, since tissues taken at this time may show sporozoites that have recently entered the host cells.

Sporozoites of this species are small compared with similar stages found in other *Eimeria*. They attain a small, spherical structure six hours after being freed from the oöcyst and measure 2.8 μ in diameter. They possess a large granule, the "hyaline globule' of some authors, and a smaller one, which is perhaps the karyosome of an indistinct nucleus. Both of these stain black with iron haematoxylin.

Sporozoites (Figs. 3 & 4) that have just entered the epithelium, elongate slightly, causing the two granules to move further apart. All of these forms are now 2.5 to 3.5 μ in length and occur mostly in the distal or lumen end of the host cell, although some have migrated past the nucleus toward the cell base. Twelve hours later many more of the developing schizonts are located in the proximal or basal region of the cell (Fig. 4).

These stages (Figs. 4 & 5), have now become 3.0 to 5.7 μ in diameter, with many showing an increase in their number of nuclei. The nuclear membranes are indistinct, comparing favorably with the observations of Tyzzer (1929) in his work on the coccidiosis of chickens.

The stages which remain on the distal side of the nucleus, develop into a generation of small merozoites.* These pockets are small and contain few individuals. The ones at the base of the cells are larger and many more merozoites are contained in each pocket. Both of these schizonts mature at the same time. To avoid confusion, the smaller ones which are also fewer in each cell are designated as type *A*

TABLE 1.—*Summary of relationships of the merozoites in four species of intestinal coccidia of the domestic rabbit*

	Species											
	E. irresidua (9–10)*			*E. magna* (7)*			*E. media* (6)*			*E. perforans* (5)*		
	Size (μ)	Days dev.	No. in 1 schizont	Size (μ)	Days dev.	No. in 1 schizont	Size (μ)	Days dev.	No. in 1 schizont	Size (μ)	Days dev.	No. in 1 schizont
1st generation type *A*	1.7 × 7.0	6	2– 8	2.8 × 8.5	4	2–10	1.5 × 6.7	4	2–10	1.5 × 5.7	4	2– 8
2nd generation type *A*	2.0 × 7.2	9	8–10	3.0 × 10.0	6	4–16	2.0 × 8.0	6	2–10	1.4 × 5.7	5	2– 8
1st generation type *B*	1.8 × 11.5	6	36–48	2.0 × 8.5	4	40–60	1.4 × 4.6	4	12–36	1.0 × 4.3	4	8–32
2nd generation type *B*	1.3 × 7.2	9	48–76	1.5 × 7.2	6	48–78	1.2 × 4.3	6	24–36	1.2 × 4.3	5	16–30

* Indicates the days required for completion of the life cycles.

* Table 1 gives in tabular form a summary of the relationship of all the species discussed in this paper.

Eimeria irresidua

FIG. 1. A sporozoite drawn from a smear six hours after infection, 2.8 μ.
FIG. 2. Sporozoites after entering a host cell, six hours.
FIG. 3. Young schizonts in the proximal and distal portions of cell, 3–4.3 μ.
FIG. 4. Young schizonts in the proximal region of a cell, 3 μ, 12 hours.
FIG. 5. Young schizont showing nuclear structure, 5 μ, 24 to 36 hours.
FIG. 6. Young schizont showing nuclear structure and food vacuoles, 7 μ, 80 hours.
FIG. 7. Young schizont, 10 μ, four days.
FIG. 8. Developing schizont of 1st generation, type *A*, 7.2×8.5 μ, six days.
FIG. 9. Immature merozoites or type *A*, pocket 5×10 μ, six days.

and the larger ones as type *B*. As schizonts designated as type *B* grow, many push past the basement membrane with their host cells bulging into the subepithelial layers.

Both types of schizonts, *A* and *B*, continue to grow rather slowly when compared with species in other animals. After 36 hours one finds them 4.5 to 7.2 μ in diameter (Fig. 5), each containing from 2 to 6 nuclei, karyosomes, and the hyaline globule.

During the 4th day one observes that the two types of schizonts exhibit signs of differentiation (Figs. 6, 7, 8, 11, 12 & 13), the ones in the distal end of the cells, the type *A*, being smaller and containing fewer nuclei than the type *B*.

After the 6th day mature merozoites (Figs. 9, 10, 14 & 15) are seen in both locations and are interpreted as representing the 1st generation, since they mature approximately at the same time. Mature pockets in the epithelium on the distal end (*A*) develop into schizonts containing from 2 to 10 banana-shaped individuals, pointed at both ends, with four as the average number present.

These merozoites are 1.5 to 1.8 μ in width by 6.5 to 7.2 μ in length. The nucleoplasm is clear except for two dark granules, one being slightly larger than the other. The nucleolus is bounded by a distinct membrane.

Between the 4th and 6th days *B* schizonts increase in size and show a multiplication of their nuclear elements. When mature (Figs. 14 & 15) these pockets are 13 by 17 μ in size and contain from 36 to 48 merozoites. These curved merozoites average 1.8 by 11.5 μ in their measurements. In the pointed extremity of most of these one finds small, darkly staining granules, while the blunt end is devoid of any such structure. The nucleus is somewhat excentrically situated toward the pointed extremity and has a prominent karyosome and a definite nuclear membrane.

The liberation of these merozoites is made possible by a gradual necrosis of the tip of the villi. These individuals are then free to produce their 2nd generation schizonts.

The developing 2nd generation merozoites derived from the 1st generation schizont *B* are located in regions similar to those for the early macrogametocytes.

The other generation of merozoites, developing at the same time as those above, are much more common and persist to the 10th day of infection. Their earlier stages (Figs. 16 & 17) present pockets showing 4 to 30 nuclei, each containing a delicate karyosome surrounded by a clear area and an indistinct limiting membrane. Later stages (Fig. 18) indicate that merozoites develop from a central mass of cytoplasm. The mature pockets contain from 48 to 75 merozoites (Figs. 19 & 20) measuring 1.3 by 7.2 μ.

Early stages (Fig. 23), showing 2 to 4 karyosomes, are found on the 6th day, and by the 7th to 8th day, one finds pockets showing 8 to 12 nuclear divisions (Fig. 24). The dense nuclear or karyosome material in the more developed schizonts

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Fig. 10. Mature merozoite of 1st generation, type *A*, 1.5–1.8 × 6.5–7.2 μ, six days.

Fig. 11. Young schizont showing nuclear differentiation, 7.5 μ, 80 hours.

Fig. 12. Young schizont showing differentiation, 8.5 μ, four days.

Fig. 13. Greatly enlarged schizont of 1st generation, type *B*, located in subepithelial area, six days.

Fig. 14. Cross section of mature merozoites of the 1st generation, type *B*, pocket 14 μ, approximate number 36, six days.

Fig. 15. Mature merozoites of 1st generation, type *B*, a small residual mass is present, 1.8 × 11.5 μ, six days.

Fig. 16. Early 2nd generation schizont, type *B*, 5.7 × 8.5 μ, eight days.

Fig. 17. Second generation schizont, type *B*, 10 × 15.7 μ, eight days.

Eimeria irresidua

FIG. 18. Immature 2nd generation schizonts, type *B*, pocket 10×14 μ, nine days.

FIG. 19. A pocket of mature 2nd generation merozoites, type *B*, individuals 1.3×7.2 μ, 9 to 10 days.

FIG. 20. Cross section of mature 2nd generation merozoites, type *B*, pocket 13×18.5 μ, number 78, 9 to 10 days.

FIG. 21. A merozoite of type *A*, 1st generation, after entrance into a new host cell, six days.

FIG. 22. Young schizont, 2nd generation, type *A*.

seem to have a spindlelike arrangement extending from one pole out into the clear area surrounding the centriole. This extension, which gives it a brushlike appearance, is finely granulated in places and corresponds to the description given by Tyzzer (1929) of *E. tenella* for a growing 2nd generation schizont.

The mature merozoites (Figs. 26 & 27) of this generation are only slightly curved, measure 2.0 by 7.2 μ, are pointed at both ends and have a nucleus situated slightly excentrically. These pockets occur less frequently than do those of type *A* and contain from 8 to 16 merozoites.

As young microgametes undergo division, the entire gametocyte increases in size. By the 8th day microgametocytes (Fig. 28) are easily distinguished from the early developing merozoites. These developmental stages of the nuclei are large, diffuse masses connected by strands and are evenly distributed throughout the gametocyte. By the 9th day metamorphosis of the microgametocytes is nearing completion; pockets containing mature microgametocytes (Figs. 29 & 30) are present, and are quite evenly distributed.

Since early macrogametocytes (Figs. 32 & 33) are found in host cells closely associated with the liberated *B* merozoites, it appears that oöcysts are derived from type *B* of the 1st generation. Later young oöcysts occur at the base of the epithelial cells in close association with connective tissue elements as were the 1st generation type *B* merozoites. After the 8th day the gametes and merozoites do not appear to be limited to any particular region of the mucosa or upper submucosa.

Early macrogametocytes (Fig. 32), 3.5 to 4.5 μ, are spherical and contain a central karyosome and possess an indefinite nuclear border with little cytoplasm. By the beginning of the 8th day (Fig. 34) they are much larger, with the average diameter 14 to 16 μ. Their cytoplasm is granular and contains a nucleus 2.8 to 3.0 μ with a large karyosome, surrounded by a clear area.

During the 8th and 9th days well developed oöcysts (Fig. 35) are seen. Each has a centrally located nucleus and surrounding cytoplasm which fills the entire cell. At the periphery are globules of material which aid in forming the cyst wall. A section through the micropyle shows the typical character of these oöcysts of *E. irresidua* (Figs. 35 & 36).

Eimeria magna

Eimeria magna, Perard (1925a), is the only one of the four species which has not been found to develop in the upper small intestine. It favors the middle jejunum and all of the ileum, requiring seven days to pass through its endogenous period.

Twelve hours after an infective feeding of oöcysts sporozoites (Figs. 37 & 38)

FIG. 23. Early 2nd generation schizont, type *A*, showing division stages, six days.
FIG. 24. Second generation schizont, type *A*, pocket 8.5 × 11.5 μ, eight days.
FIG. 25. Second generation schizont, type *A*, division stage present, pocket 11.5 μ, eight days.
FIG. 26. Nearly mature 2nd generation, type *A*.
FIG. 27. Mature 2nd generation, type *A*, merozoites, individuals 2 × 7.2 μ, nine days.
FIG. 28. Young microgametocyte showing diffuse masses still in the process of division, pocket 18 × 28 μ, eight days.
FIG. 29. Microgametocyte showing young microgametes, pocket 15.7 × 18.5 μ, nine days.
FIG. 30. Microgametocyte showing mature microgametes, pocket 20 × 28.5 μ, nine days.
FIG. 31. Microgametes × 2700.
FIG. 32. Young macrogametocyte, 4.3 μ, six days.
FIG. 33. Young macrogametocyte, 4.3 × 5.7 μ, six days.
FIG. 34. Growing macrogametocyte, 14 × 16 μ, eight days.

Eimeria irresidua

FIG. 35. Macrogametocyte ready to be discharged from host cell, 24 × 36 μ, nine days.
FIG. 36. Fully sporulated oöcyst.

Eimeria magna

FIG. 37. Sporozoite from an intestinal smear 12 hours after an infective feeding.
FIG. 38. Young schizont, 2.0 μ, 12 hours.

are found to have just entered their host cells. Young schizonts (Figs. 39, 40, & 41) are found by the 36th hour of infection. These larger schizonts show 1 to 2 nuclei and average 3.5 μ in diameter. During the next 24 hours the schizonts double in size as the nuclei increase in number (Fig. 42) and by the 3rd and 4th days still larger schizonts are observed showing continued nuclear divisions (Figs. 43 & 44). On the 4th day young and adult merozoites will be seen which belong to two distinct types previously mentioned as occurring in *E. irresidua*. Both of these types should be considered 1st generation merozoites. Schizonts of the type *A* (Figs. 45 & 46) merozoites measure 10 by 14 μ, and contain from 2 to 10 individuals which are 2.8 by 8.5 μ when mature. They are curved, pointed at one end and blunt at the other, with a nucleus that is usually centrally located. These merozoites immediately enter other host cells to become either 2nd generation schizonts or develop into gametes. The 2nd generation schizonts soon begin nuclear division if they are to undergo schizogony and become 2nd generation merozoites.

The other types of merozoites which are here designated as *B*, 1st generation (Figs. 48–51), are smaller, containing from 40 to 60 in a pocket; they are slightly curved, pointed at one end and rounded at the other, and measure 2.0 by 8.5 μ. These have a central nucleus with a pronounced clear area about the pole toward the rounded end. They may produce either gametes or another generation of merozoites.

Fig. 52 shows, what appears to be, a rounded merozoite in a host cell which probably develops into the generation of merozoites which is destined as 2nd generation type *B*. On a slide prepared after 5 to 6 days infection one finds schizonts containing 6 to 75 immature merozoites (Figs. 53 & 54). More advanced merozoites are seen in association with the central mass (Fig. 55) as well as mature ones (Fig. 56). Pockets containing these adult individuals with their components measure 19.5 by 21.4 μ.

Figs. 57–62 show phases in the progressive maturation of 2nd generation *A* schizonts containing few, large individuals. These, when mature, average maximum lengths of 3.0 by 10 μ. They are pointed at one end and blunt at the other with a

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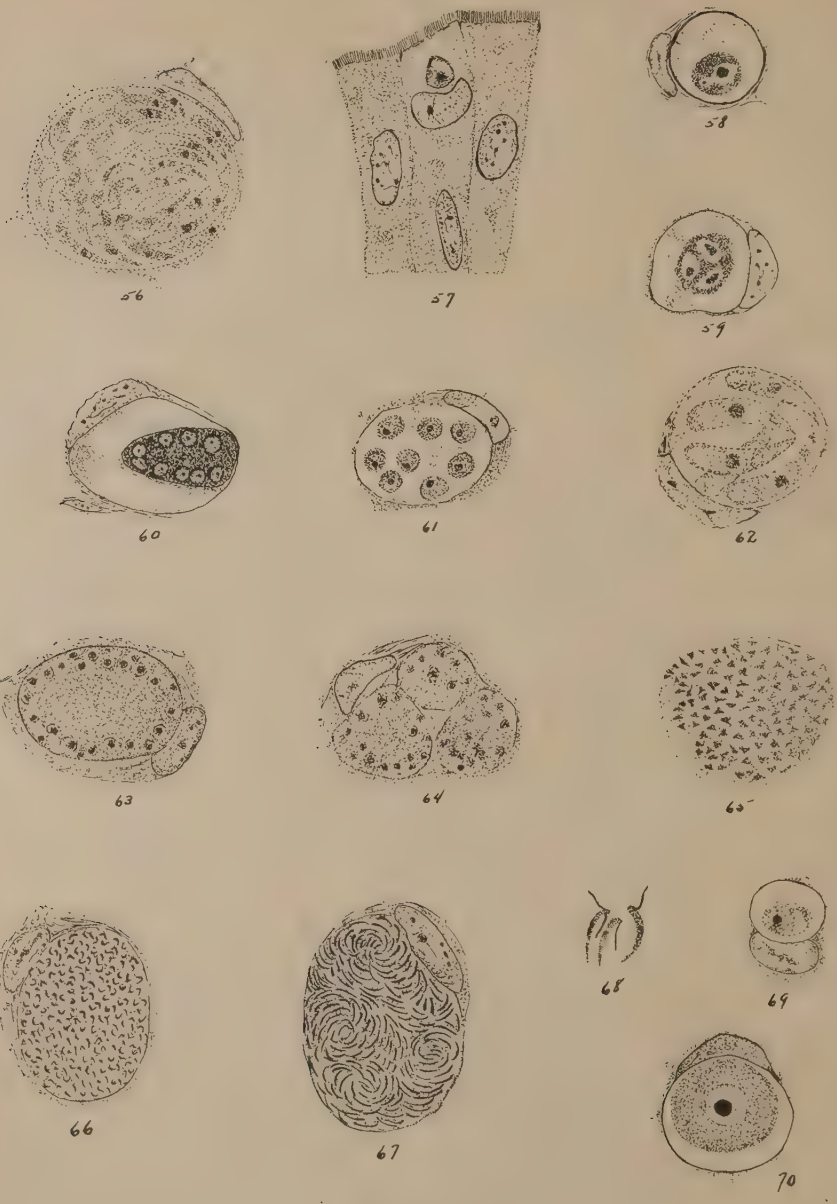
Fig. 39. Young schizont, 2.8 μ, 24 to 36 hours.
Fig. 40. Young schizont, 3.5 μ, 36 hours.
Fig. 41. Young schizont, 3.5 μ, 36 hours.
Fig. 42. Young schizont showing nuclei, 4.3 μ, 60 hours.
Fig. 43. Young schizont showing nuclei, 7.5 μ, 80 hours.
Fig. 44. Young schizont, 1st generation, type *A*, 7.2 × 10 μ, four days.
Fig. 45. Mature merozoites, 1st generation, type *A*, individuals 2.8 × 8.5 μ, four days.
Fig. 46. Cross section of pocket containing six merozoites of 1st generation, type *A*, pocket 10 × 14.4 μ, in six days.
Fig. 47. A 1st generation, type *A*, merozoite, 2.8 × 8.5 μ, four days.
Fig. 48. Young schizont of 1st generation, type *B*, 13 × 21.5 μ, 3 to 4 days.
Fig. 49. Young schizont of 1st generation, type *B*, showing one of the merozoites of elongation, 18 μ, four days.
Fig. 50. A pocket of nearly mature merozoites, 17 × 24 μ, four days.
Fig. 51. Mature 1st generation, type *B*, merozoite 2.0 × 8.5 μ, four days.
Fig. 52. A 1st generation, type *B*, merozoite after entering a new host cell, destined to become a 2nd generation, type *B*, schizont, 2.8 μ, four days.
Fig. 53. Second generation, type *B*, schizont, pocket 8.5 × 10 μ, five days.
Fig. 54. Second generation, type *B*, schizont showing immature merozoites coming from a central mass, 13.0 × 18.5 μ, six days.
Fig. 55. Young merozoites of 2nd generation, type *B*, some are still clinging to a central mass, pocket 17.0 μ, six days.



Eimeria magna

FIG. 56. Mature pocket of 2nd generation, type *B*, merozoites, pocket 18.5×21.4 μ, individuals, 1.5×7.2 μ, six days.

FIG. 57. Young schizont of 2nd generation, type *A*, six days.

FIG. 58. Young schizont, type *A*, showing 1st division, six days.

FIG. 59. Young schizont, type *A*, showing three nuclear structures, 5.7×7.2 μ, six days.

FIG. 60. Young schizont, 2nd generation, type *A*, showing eight nuclear divisions, surrounding a central mass, 7.2×13 μ, six days.

centrally located nucleus. There are from 4 to 16 individuals in a pocket. Merozoites of this type are the largest encountered in any of these species.

It is impossible to distinguish the early gametes from one another, but on the 5th day, one observes stages (Fig. 63) which are destined to become microgametocytes. In these pockets the nuclear structures undergo a large number of divisions while the cytoplasm increases in volume. These small nuclear stages (Fig. 64) are but 1 μ in size and appear more definite than in other species. During their transformation they pass successively from triangular (Fig. 65) to comma-shaped individuals (Figs. 66 & 67), measuring 0.5 by 2.5 to 3.0 μ. Many of these microgametocytes appear lobulated, caused by an infolding of their surfaces (Fig. 64). The fully developed microgametocytes appear in whorls and are quite evenly distributed throughout the gamete although in some pockets they tend to collect toward the periphery. Mature microgametocytes show terminal flagella, and what appears to be a nucleus (Fig. 68) located in the mid-region.

The early, spherical macrogametes (Fig. 69) are definitely recognized from the 5th to 6th day; the older of these (Fig. 70) possesses a nucleus which contains a prominent karyosome which is surrounded by a fine, granular nucleoplasm and a relatively distinct nuclear membrane. Large, spherical forms (Fig. 71), which measure 18 by 20 μ, contain large plastic globules in their cytoplasm. These are the periphery and elongate as their walls are formed and begin to assume the characteristic morphologic appearance of *E. magna* (Fig. 73) with its typical micropyle. These intracellular stages are 15 to 20 by 30 to 35 μ in diameter.

Eimeria media

The endogenous phases of the intestinal parasite, *Eimeria media* Kessel and Jankiewicz (1931) take place in the epithelium throughout the entire small intestine. Tissues taken after an infection of from 12 to 24 hours show young schizonts (Fig. 74) in the process of development; some are located distally in the cells while others are located in the proximal regions. During the 2nd day after infection the schizonts (Fig. 75) have increased in size, measuring 5 μ in diameter and showing on an average six nuclei distributed throughout their cytoplasm. This species exhibits, in its earlier stages, the characteristic hyaline globule that is found in other species. During the next 36 hours the schizonts continue to grow and differentiate into type *A* and *B* merozoites of the 1st generation. On the 4th day one finds early

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Fig. 61. A cross section of a mature pocket of 2nd generation, type *A*, merozoites, pocket, 10 × 14.4 μ, six days.

Fig. 62. Mature merozoites of type *A*, 3.0 × 10 μ, these sometimes exhibit a filamentous structure on their narrow extremity, 3.0 × 10 μ, six days.

Fig. 63. Young microgametocyte showing many rounded nuclear stages, 10 × 16 μ, five days.

Fig. 64. Later stage of microgametocyte showing infolding of the cytoplasm, 15 × 17 μ, five days.

Fig. 65. Microgametocyte showing triangular stages of the microgametes, six days.

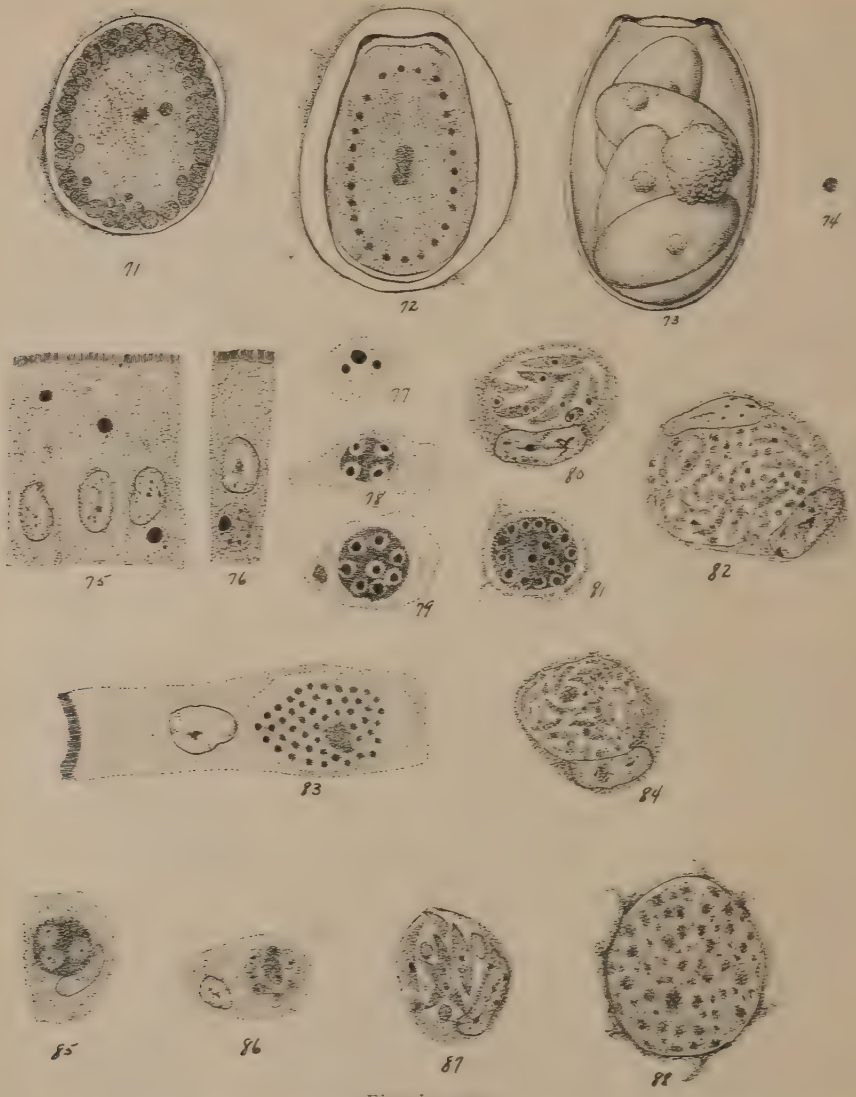
Fig. 66. Microgametocyte showing elongation of the immature microgametes, 14.4 × 23 μ, six days.

Fig. 67. Mature pocket of microgametes showing whorl arrangement, pocket, 14 × 24 μ, individuals, 2.5 × 3.0 μ, seven days.

Fig. 68. Several mature microgametes showing what appear to be nuclei in their mid regions, × 2700.

Fig. 69. Young macrogametocyte, 3.0 × 4.3 μ, five and six days.

Fig. 70. Older macrogametocyte, 10 μ, six days.



Eimeria magna

FIG. 71. Large, spherical macrogametocyte showing "refractile globules" at periphery of cytoplasm. 18 × 20 μ, six days.

FIG. 72. Macrogametocyte showing characteristic micropyle. 15 × 30 μ, seven days.

FIG. 73. Fully sporulated oöcyst.

Eimeria media

FIG. 74. Sporozoite from an intestinal smear, 2.0–2.3 μ, 12 hours.

FIG. 75. Young schizonts, 2.0–3.5 μ, 24 hours.

FIG. 76. Older schizont showing several nuclei, 5 μ, two days.

FIG. 77. First generation schizont showing larger nuclei, 6 μ, two days.

FIG. 78. Young 1st generation type of schizont showing four nuclear structures, 5 × 4 μ, 3 to 4 days.

stages which represent immature stages of the type destined to become the type *A* merozoites (Figs. 77 & 78). Also at this same time young schizonts (Fig. 79), showing as many as eight nuclear divisions, are present; these nuclear masses stain intensely with iron haematoxylin and are surrounded by clear areas. These structures seem to develop from a common cytoplasmic mass.

Mature merozoites (Fig. 80) derived from these schizonts are present in the same tissue with the previous forms. These merozoites (Fig. 80) derived from these schizonts are present in the same tissue with the previous forms. These merozoites are, for the most part, slightly curved, pointed at both ends and contain a centrally located nucleus which is surrounded by a clear area. They measure 1.5 by 6.0 μ and are found in pockets containing from 2 to 10 individuals.

Earlier schizonts (Fig. 81) which are the forerunners of type *B* of the 1st generation, are seen during the 4th day. They measure 7.2 by 8.4 μ and contain many nuclear divisions all embedded in a dark, cytoplasmic material. At this time are found small, mature merozoites (Fig. 82) which are slightly curved, pointed at both extremities and contain a centrally located nucleus. These measure 1.4 by 4.6 μ and number from 12 to 36 individuals in each pocket.

The early 2nd generation type *B* merozoites (Fig. 83), show many nuclear divisions; they are contained in pockets which measure 10 by 17 μ. The adults (Fig. 84), of this type, are 1.2 by 4.3 μ and are contained in pockets measuring 11.5 by 13 μ. This type of merozoites does not cause as much hypertrophy of its host cells as do those of *E. irresidua* and *E. magna;* they closely resemble *E. perforans* in this respect.

The 2nd generation type *A* merozoites appear on the 6th day and are represented by small schizonts (Fig. 85) containing extremely small nuclei. Later stages (Fig. 86) also appear at this time, showing five or six nuclear divisions, contained in pockets measuring 5.7 by 7.2 μ. Mature merozoites (Fig. 87) measure 2.0 by 8.0 μ, from 2 to 8 individuals occurring in each pocket. They are pointed at both extremities and exhibit a centrally located nucleus.

The metamorphosis of microgametes seems to be similar to that encountered in other species. On the 5th and 6th days one observes microgametocytes (Fig. 88) containing the typical, diffuse nuclear material as well as other stages in which it is condensed (Fig. 89). Pockets containing mature microgametes (Fig. 90) measure approximately 17 μ in diameter with individuals measuring about 2 μ in length. The mature microgametes (Fig. 91) have only an indication of a nucleus but do possess flagella.

Macrogametocytes (Fig. 92) also appear on the 5th and 6th days. These are

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Fig. 79. First generation schizont, type *A*, showing eight nuclear divisions, 7.5 μ, four days.
Fig. 80. Mature 1st generation, type *A*, merozoites, 1.5 × 6–7.0 μ, four days.
Fig. 81. Early 1st generation, type *B*, schizont, 7.2 × 8.5 μ, four days.
Fig. 82. Mature 1st generation, type *B*, merozoites, pocket, 18 × 20 μ, individuals, 1.4 × 4.6 μ, four days.
Fig. 83. Young 2nd generation, type *B*, schizont, 10 × 17 μ, six days.
Fig. 84. Pocket of 2nd generation, type *B*, merozoites, pocket, 70 × 21 μ, individuals, 1.2 × 4.3 μ, six days.
Fig. 85. Young 2nd generation, type *A*, schizont, 5.7 μ, 5 to 6 days.
Fig. 86. Older stage of developing 2nd generation, type *A*, merozoites, 5.7 × 7.2 μ, six days.
Fig. 87. Pocket of mature 2nd generation, type *A*, merozoites, 2.0 × 8.0 μ, six days.
Fig. 88. Early microgametocyte, 21 μ, six days.

Eimeria media

Fig. 89. Later stage of a microgametocyte, six days.
Fig. 90. Mature microgametocyte, 17.0 μ, individual microgametes, 2.0 μ, six days.
Fig. 91. Mature microgamete, × 2700.
Fig. 92. Young macrogametocyte, 5.7 μ, six days.
Fig. 93. Older macrogametocyte, 14.4 μ, six days.
Fig. 94. Macrogametocyte ready to be liberated, 14.4 × 25.7 μ, six days.
Fig. 95. Fully sporulated oöcyst.

small, spherical structures exhibiting the typical dark karyosome. Later stages (Fig. 93) present the usual refractile globules which are lost as the oöcyst wall develops. Oöcysts, ready to be discharged from the host cell, show a zygote surrounded by an evenly staining cytoplasm which fills the entire cyst. This intracellular stage measures 14 by 25 μ. Sporulated oöcysts (Fig. 95) of this species are more variable in their length-breadth ratio than any of the others.

Eimeria perforans

Eimeria perforans Leuckart (1879), is the smallest known parasite of the intestinal group, and has a developmental cycle ranging from five to five and one-half days. It is distributed from the duodenum to the lower ileum.

Sporozoites (Fig. 96) are found free in the lumen a few hours after an infective feeding. They are small, darkly staining, spherical structures 2 μ in diameter. Tissues taken after an infective period of 12 hours, show sporozoites (Fig. 97) that have just entered the epithelium as well as young schizonts, some distal to the nucleus. During the next 12 hours the schizonts (Figs. 98 & 99) double in size while their nuclei increase to three or four. On the 2nd day still larger schizonts are observed showing divisions in their cytoplasm and the presence of six or eight nuclei (Fig. 100). During the later 3rd and early 4th days first generation merozoites are found, which are considered type *A*. The pockets (Figs. 101 & 102) containing these measure 5.7 to 10 μ and contain 4 to 8 individuals which measure 1.5 by 7.2 μ. These are blunt at both ends with a delicate nucleus surrounded by a clear area all located more toward one end than the other.

On the 3rd day of infection, type *B* forms are first distinguished. These stages (Fig. 103) show, on an average, 15 nuclear divisions. During the 4th day these mature and the small pockets show from 8 to 24 merozoites. These are the smallest merozoites (Figs. 104 & 105) encountered in this study; the individuals, measuring 1.0 by 4.3 μ, are contained in pockets measuring 4.3 by 5.7 to 7.0 by 8.0 μ. For the most part they are pointed at both extremities with their nuclei located approximately in the central region.

Intermediate stages in type *B* of the 2nd generation (Fig. 106) have been difficult to locate in this study. Mature pockets of these contain, on an average, 24 mero-

Eimeria perforans

Fig. 96. Sporozoite from an intestinal smear, 2 μ, 12 hours.

Fig. 97. Four epithelial cells showing sporozoites after entering the host cells and young schizonts, 12 hours.

Fig. 98. Schizonts showing nuclei, 4 μ, 24 hours.

Fig. 99. Schizont, 4.3 μ, 24 hours.

Fig. 100. Early schizont of the 1st generation, type *A*, showing cytoplasmic division, 5.7 × 8.5 μ, two days.

Fig. 101. Mature merozoites of the 1st generation, type *A*, individuals, 1.5 × 5.7 μ, four days.

Fig. 102. Mature merozoites of type *A*, four days.

Fig. 103. Early schizont of 1st generation, type *B*, 4.3 × 5.7 μ, three days.

Fig. 104. Schizont of 1st generation, type *B*, merozoite, also a young gametocyte is shown, four days.

Fig. 105. Pocket of mature 1st generation, type *B*, merozoites, 7.8 μ, individuals, 1.0 × 4.3 μ, four days.

Fig. 106. Developing schizont, 2nd generation, type *B*, 7.2 μ, four days.

Fig. 107. Mature merozoites of the 2nd generation, type *B*, individuals, 1.2 × 4.3 μ, five days.

Fig. 108. Early schizont of 2nd generation, type *A*, 4 μ, four days.

Fig. 109. Young schizont of the 2nd generation, type *A*, 5.7 μ, five days.

Eimeria perforans

FIG. 110. Young schizont of type *A* in which the divisions are nearly complete, five days.

FIG. 111. Cross section of a pocket of mature 2nd generation, type *A*, merozoites, 5.7 μ, five days.

FIG. 112. Mature 2nd generation, type *A*, merozoites, individuals, 1.4 × 5.7 μ, five days.

FIG. 113. Several cells showing stages which result after the entrance of 2nd generation, type *A*, merozoites.

zoites which measure 1.2 by 4.0 μ and have a centrally located nucleus. It is not uncommon, in these mature pockets (Fig. 107) to find groups of merozoites arranged in clusters in one end of the pocket while in the other regions they appear more diffuse in their arrangement. This may result from the development of two schizonts in one host cell.

Type *A* merozoites, when liberated, enter other epithelial cells and from young schizonts (Figs. 108 & 109) which become 2nd generation merozoites, type *A*. The pockets of these, which are mature on the 5th day, contain 4 to 8 crescentic individuals (Fig. 110). Their nuclei are large, centrally located and surrounded by a clear area. During development, this generation can be recognized by the density of its chromatin material. These merozoites (Figs. 111 & 112) continue the endogenous cycle as is shown in Fig. 113 which exhibits merozoites penetrating into new host cells to form new schizonts.

Microgametocytes occur during the 4th to 5th day after infection. The earliest stages (Fig. 114) exhibit a diffuse chromatin material. The pockets measure 7.2 by 10 μ. Later stages show small, triangular microgametes (Fig. 115) while the mature microgametes (Fig. 116) are typical, comma-shaped structures which measure approximately 2 μ in length; they are contained in pockets measuring 13 by 17 μ. These microgametes also show a darkly staining protoplasmic mass which suggests a nucleus.

Small macrogametocytes (Fig. 117) are usually observed on the 4th and 5th days. Their development is similar to that described for other species except that they are smaller (Figs. 118 & 119).

E. perforans appears to be the least pathogenic of any of the intestinal species. This is probably due to the production of fewer merozoites.

Frequently the mature oöcyst (Fig. 121) fails to exhibit a micropyle until several days after sporulation is apparently complete.

DISCUSSION

It is now generally recognized that there are five species of *Eimeria* occurring in the rabbit. One of these, *E. stiedae* is found in the liver while the other four are found in the small intestine. Any combination of the four intestinal species may be found in the naturally infected rabbit and can be readily differentiated from each other by a study of the oöcysts. After obtaining the oöcysts of each of the four species in pure form, infecting suitable animals with large doses, sacrificing the animals at appropriate times and preparing histological sections, it is possible to study the endogenous cycles of these *Eimeria*. The author, in this investigation, confirms

FIG. 114. Young microgametocyte, 7.2 × 10 μ, five days.
FIG. 115. Immature microgametocyte, 10.0 × 15.7 μ, five days.
FIG. 116. A microgametocyte with mature microgametes; some free. Microgametocyte, 13.0 × 17.1 μ, microgametes, 2 μ. Neighboring cell shows an aberrant form.
FIG. 117. Mature microgametes, × 2700.
FIG. 118. Young macrogametocyte, 5.7 μ, 4 to 5 days.
FIG. 119. Older macrogametocyte, 11.5 μ, five days.
FIG. 120. Maturing macrogametocyte, 9.0 × 17.5 μ, five days.
FIG. 121. Fully sporulated oöcyst.
FIG. 122. Undeveloped oöcyst of *E. irresidua*.
FIG. 123. Undeveloped oöcyst of *E. magna*.
FIG. 124. Undeveloped oöcyst of *E. media*.
FIG. 125. Undeveloped oöcyst of *E. perforans*.

the findings of Kessel and Jankiewicz (1931) by a study of the endogenous phases of these four species. The length of time for completion of these cycles is as follows: *E. irresidua,* 9 to 10 days; *E. magna,* seven days; *E. media,* six days; and *E. perforans,* five days.

Perard (1925a) does not give the length of time required for the endogenous cycle of *E. magna* but states that the schizonts of *E. magna* are oval or spherical measuring 10 to 25 μ with merozoites variable in size measuring 1.0 to 2.5 by 4.0 to 10.0 μ and says that from 2 to 50 merozoites may be present in each schizont. From the above data it appears that he was working with a mixed infection of *E. perforans* and *E. magna.* The larger merozoites reported in his paper (2.5 to 10 μ) correspond favorably with those given here for *E. magna* (3.0 to 10 μ) which occur in the type *A* 2nd generation. The size of the schizonts and the number of merozoites reported by him from each schizont correspond to those occurring in either of the type *B* schizonts of the 1st or 2nd generation. The smaller merozoites he reports (1.0 by 4.0 μ) are similar to those found to exist in this work for *E. perforans* (1.0 to 1.2 by 4.3 μ).

In studies on *E. perforans,* Perard reports they have a life cycle requiring four days and develop into a mature spherical schizont measuring 15 μ in diameter and contain "from 30 to 70 long, slender, spindle-shaped, slightly-bowed merozoites, arranged side by side, and each measuring from 10 to 12 μ in length and from 0.5 to 1.75 μ in breadth." Mature schizonts of *E. perforans* in this study measure 4.3 to 10 μ, this size depending upon the type of merozoites contained therein. Merozoites for type *A* of the 1st generation are 1.5 by 5.7 μ and those of type *A* 2nd generation measure 1.5 by 6.0 to 7.0 μ, and from 2 to 8 merozoites develop in each cell. The 1st generation type *B* merozoites measure 1.0 by 4.3 μ and contain from 8 to 32 in each cell, 20 being the average number. The 2nd generation type *B* measure 1.2 by 4.3 μ, containing 20 to 30 in each cell. Merozoites of the size reported by Perard (0.5 to 0.75 by 10 to 12 μ) have not been found in any of this work, and nothing is mentioned in his work which would indicate the types of merozoites that have been found to occur in this study.

Within a few hours after infecting rabbits with any of these several species, one finds sporozoites free in the lumen and in the intestinal epithelium. These sporozoites seem to remain in an infective condition in the intestine for as long as two days since tissues taken at this time show sporozoites just inside the epithelium. This actually means that younger schizonts are found intermingled with the older stages in the host cells.

The sporozoites encountered on intestinal smears are all small, rounded individuals probably being composed of only nuclear material since no cytoplasm is observed.

Schizonts derived from these develop slowly when one compares them with similar developmental stages in other animals, for example: in *E. irresidua,* which has a cycle of 9 to 10 days, no 1st generation merozoites appear until five or six days after infection while in *E. separata* from the rat, as reported by Roudabush (1937), the 1st generation merozoites are completely formed after 24 hours.

In all species 1st generation schizonts develop in the epithelial cell. When the 1st generation merozoites are liberated they appear to be of two distinct types and are both liberated at approximately the same time. Because this occurs, it is considered

necessary in this work to designate them as type *A* and type *B* of the 1st generation. Type *A* always contain fewer individuals, while type *B* contains many more, except in *E. irresidua* where type *A* merozoites are fewer than type *B*. The size of the merozoites has been shown by other authors but they have failed to designate them as two distinct types.

Merozoites of these types form gametes or 2nd generation merozoites which are designated as *A* and *B* on the basis of size. Second generation merozoites complete their development in from 1 to 3 days depending upon the species.

Gamete formation begins when the 1st generation merozoites enter the host cells. The young gametes are not readily distinguished until the division of their nuclei is discernible. All young gametocytes consist of a central karyosome around which appears a clear area which in turn is surrounded by an area of more dense cytoplasm.

In the development of the microgametocytes, the nucleus undergoes numerous divisions and at the same time the cytoplasm increases in volume. The early chromatin masses are diffuse and connected with one another by fine threads. As development proceeds, these nuclei condense, becoming somewhat pyramidal in shape and as they reach maturity, they become roughly comma-shaped. The fully developed microgametocytes are contained in exceedingly large vesicles often surpassing the macrogametocyte in size but their number is far less.

Young oöcysts are spherical, containing a centrally located karyosome. The cytoplasm of the macrogametocyte contains refractile globules which gradually accumulate at the periphery. These probably serve as reserve food material which aids in the formation of the oöcyst wall. All oöcysts, before their liberation, exhibit a finely granulated cytoplasm and their characteristic micropyle. After the oöcyst is discharged from its exhausted host cell into the lumen, its cytoplasmic mass, which is now the zygote, assumes a spherical shape until discharged with the feces. Outside of the host, under favorable conditions, it will sporulate.

All species, with the exception of *E. magna*, complete their cycles throughout the length of the small intestine. *E. magna* apparently does not inhabit the upper 12 inches of the gut. *E. irresidua* seems to produce the 1st generation of merozoites only in the first 18 to 24 inches of the gut; after liberation of the 1st generation of merozoites, the cycle is completed throughout the entire length of the intestinal tract.

Animals given heavy infective doses of oöcysts show no outward symptoms until approximately 24 hours before the oöcysts are to be discharged, at which time they refuse food and water and appear inactive. These heavy infections give rise to a severe diarrhea which lasts about 12 hours, often resulting in the death of the animal.

E. irresidua and *E. magna* produce the most pronounced pathological changes in the intestinal epithelium. This is probably due to their larger size and greater fecundity as well as their ability to penetrate deeper into the glands while the others remain more in the surface regions and do not seem so prolific. All of these parasites develop within the epithelial cells of the mucosa causing a necrosis of most of the parasitized cells.

The growth of the stages in the cells gradually causes the host cell to enlarge in order to accommodate the parasite. Sometimes the cytoplasm appears only as an outline while in other cases it maintains a considerable volume. The nuclei hyper-

trophy to as much as twice their normal size and appear to be the last cell structure to degenerate.

E. irresidua is the only one of these species which penetrates to the submucosal layers. Epithelium containing stages of parasites are often sloughed into the lumen. This appears to be accomplished by a constriction occurring between the parasitized region and the regenerating epithelium. Denuded areas show various stages of necrosis with the underlying tissues exhibiting active hyperemia and some extravasation of the blood elements.

The majority of *Eimeria* are known only in the oöcyst form. Since many of these resemble one another quite closely it is necessary to rely on their distinguishing characteristics for differentiation. Some of these are measurements, shape, color, cell wall, appearance of micropyle, and the presence or absence of residual bodies.

As the life histories of these different coccidia are discovered, they substantiate the fact that these different oöcysts represent the exogenous stages of specific species.

In the chicken, six species of *Eimeria* are reported, with oöcysts ranging in size from 15.5 by 16.2 to 22.6 by 29.3 μ, while in the rabbit their range is 14.2 by 22.7 to 25.6 by 38.3 μ and in the three species of the rat the range is 16.06 by 13.85 to 24.38 by 22.12 μ. The endogenous cycle of all these *Eimeria* of the chicken and rabbit complete their development in 4 to 7 days, with the exception of *E. irresidua*, which takes 9 to 10 days, and *E. stiedae*, which requires approximately 18 days.

The *Eimeria* of the rat produce 3 to 4 generations of merozoites, while the 1st generation is liberated after an infective period of 24 to 48 hours. A generation is produced every 24 hours thereafter until schizogony is complete. In the chicken the 1st generation of merozoites is liberated between 2½ and 4 days after infection. *E. mitis* of the chicken corresponds more closely to the rabbit coccidia, since its 1st generation merozoites are liberated on the 4th day. In the rabbit the 1st generation merozoites of the intestinal species grow rather slowly and are liberated from 3½ to 6 days after infection, with two distinct types appearing simultaneously; the 2nd generation also produces two distinct types which are liberated at the same time. In *E. perforans* a period of 24 hours occurs between the liberation of the 1st generation merozoites and the production of 2nd generation merozoites. The other intestinal species require 2 to 3 days for the development of similar stages. We thus see that species of coccidia in other animals have generations of merozoites liberated at times intermediate between those required in the rabbit.

The rat has two *Eimeria* parasitizing the small intestine, while a 3rd species requires the cecum and the colon in which to complete its cycle. In the chicken, *E. tenella* has schizonts and oöcysts in the ceca, in *E. necatrix* the schizonts are in the "small intestine," and oöcysts are in the "ceca," with the others favoring the small intestine for their development. Four of the species of the rabbit favor the small intestine, while the 5th lives in the bile ducts.

The above-mentioned species of coccidia from the chicken are the most pathogenic, while in the rabbit *E. magna* and *E. irresidua* appear to cause the most severe infections. All of the *Eimeria* live only in the epithelium of endodermal origin, or in the subepithelial regions or in both locations.

SUMMARY

1. Studies of the endogenous cycles of the four species of intestinal *Eimeria*, found in the domestic rabbit, show that each produces two types of merozoites.

These we may designate as type *A* and *B*, each of which produces a 1st and 2nd generation of merozoites.

2. Merozoites of the 1st and 2nd generations of type *A* of all four species, with the exception of the 1st generation type *A* of *E. irresidua,* are large and contain from 2 to 16 merozoites in each schizont. Those occurring in the 1st and 2nd generation of the type *B* except in the 1st generation of *E. irresidua,* are small and contain from 12 to 78 merozoites in each schizont.

3. It is possible that the 1st generation of type *A* merozoites gives rise to a 2nd generation of type *A* merozoites and microgametocytes, while type *B* merozoites give rise to a 2nd generation of type *B* merozoites and macrogametocytes.

4. Merozoites of the 1st generation of both type *A* and *B* of the four species described require the following lengths of time to mature: *E. irresidua,* 5 to 6 days; *E. magna,* four days; *E. media,* four days; and *E. perforans,* four days.

5. Merozoites of the 2nd generation of both type *A* and *B* of all four species require the following lengths of time to reach maturity: *E. irresidua,* three days; *E. magna,* 2 to 3 days; *E. media,* two days; and *E. perforans,* one day.

6. The four species here reported require the following lengths of time for the completion of their endogenous cycles: *E. irresidua,* 9 to 10 days; *E. magna,* seven days; *E. media,* six days; and *E. perforans,* five days.

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A CONTRIBUTION TO THE BIOLOGY OF *ORNITHODOROS HERMSI* WHEELER, HERMS AND MEYER

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Collections to date indicate that the argasine tick, *Ornithodoros hermsi* Wheeler, Herms and Meyer (Wheeler, Herms, and Meyer, 1935; Wheeler, 1935) is apparently restricted to the mountainous regions of the western United States, specimens having been taken at elevations above 5000 feet from California, Idaho, and Colorado, and at elevations of 3000 and 3600 feet from Oregon.

HABITAT

This species of tick is a parasite of small mammals, particularly rodents, with the most favored host the western chipmunks, *Eutamias* spp. The majority of ticks in California were taken from the nesting material of these animals found in the attics and between the walls of some of the cabins and cottages in several of the mountain resort areas (Herms and Wheeler, 1935). Other specimens have been found in nesting material of chipmunks situated in old hollow logs while several were removed from the sleeping bag of a deer hunter. In Colorado many ticks of this species were collected from chipmunk nesting material in a decaying Douglas fir stump (Davis, 1941). No ticks have been discovered in the nests of the chickaree squirrel, *Sciurus douglasii*, although this species of rodent is another proven reservoir of the spirochetes of relapsing fever. That these squirrels locate their nests high in trees making search extremely difficult in all probability accounts for the fact that no ticks have been taken from the nests of these animals.

METHOD OF REARING

Ticks reared in the laboratory were individually confined in circular pasteboard pill boxes, $1\frac{1}{4}$ by $\frac{3}{4}$ inches. These were arranged in rows on a metal tray constructed from one inch angle-iron to which was soldered a sheet of $\frac{1}{4}$ inch-mesh hardware cloth forming a tray allowing free circulation of air around each of the pill boxes when placed in a temperature and humidity control cabinet (Fig. 1). Warmth was provided by a 60-watt electric lamp, and the temperature was controlled by a mercury column thermostat. Humidity was provided by means of a coarse towel suspended from a wire frame in front of a small electric fan, the free end of the towel being submersed in a shallow pan of water. A hygrothermograph placed on the floor of the chamber gave continuous accurate recordings of both humidity and temperature. After some experimentation it was determined that the ticks developed best at approximately 75° F, and with a humidity of not less than 90 per cent.

FEEDING METHOD

A narrow cylindrical feeding cage fashioned from $\frac{1}{4}$-inch-mesh hardware cloth was used to confine the mouse while the ticks fed. This cage was made by rolling a piece of the hardware cloth into a cylinder about six inches in length with both ends

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FIG. 1. Constant temperature and humidity cabinet in which ticks were reared.

open and just large enough to receive a mouse (Fig. 2). The opening was inch long and ½ inch wide and cut in the center of the cylinder to allow the head access to a portion of the experimental animal. The mouse to be used was selected and the hair over its thorax and a plug of cotton forced into the "head end" of the tube as far as the mouse was in position. A second plug of cotton was then forced into the opposite end of the tube behind the mouse. This cylinder is adjustable to the size of the mouse, and the animal can be easily immobilized by pushing the two plugs of cotton further into the cage. The hair on the mouse was then clipped exposing the area of the abdomen, and the ticks were applied on the mouse by means of fine camel's hair pen. When feeding was complete, the tick usually dropped from the animal but in some instances were removed by forceps. To illustrate the mouse and plug of

FIG. 2. Adult [illegible] ticks feeding on white mouse confined in cylinder feeding cylinder.

cotton in front was removed and the animal allowed to crawl out. This method of feeding proved quite satisfactory and allowed for easy handling and observation of the ticks under a binocular dissecting scope.

The majority of the ticks attached immediately, the period of time required for complete engorgement varied with the different instars. The feeding periods for a series of several hundred ticks in all active stages are shown in Table 1.

TABLE 1

1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar	
	Min.	*Min.*	*Min.*	*Min.*	
Did	7	11	12	10	Minimum
not	40	131	66	70	Maximum
feed	16	31	32	28	Average

OVIPOSITION

The act of oviposition, which is similar to that already observed in other species, was observed under the binocular in one instance, the adult female tick lying with the ventral surface exposed to view. The inflated, translucent, extremely thin-walled, eversible gland, Gene's organ (Samson, 1909), was noticed protruding posteriorly to and at the base of the ventral margin of the hood, between the hood and the basis capituli. This organ consists in part of two long, separable finger-like processes which, when extended, curve upward and extend over the mouth-parts, the rounded tips reaching posteriorly nearly to the genital orifice.

At this moment two eggs were observed clinging to this organ, being held in place by a viscous substance. The genital orifice was raised and drawn towards the mouth-parts by the contraction of the muscles in the region of the first pair of coxae. A third egg was observed to emerge suddenly from the genital opening; at the same time the "fingers" of the organ were extended to grasp the egg which became fastened to the other two eggs already adhering to the structure. The mouth-parts were flexed toward the genital opening in such a manner that when the organ was returned to position the mouth-parts assisted in pushing the eggs over the capitulum and hood to beyond the outer anterior margin of the body where they were added to the mass of eggs previously extruded, the egg mass being held together by the viscous material apparently secreted by the organ.

Shortly preceding the extrusion of the egg from the genital opening and continuing for a period of two minutes, a series of pulsating movements was noticed to occur under the body wall around the genital aperture. These movements continued until the organ was returned to its normal position within the body cavity. The "fingers" of the organ itself remained in an extended position during the period of egg deposition. At the end of egg deposition or when disturbed the organ slowly contracted or appeared to become deflated as the pressure of the fluid within was reduced and the fluid had receded into the body of the tick. At the same time the mouth-parts gradually assumed their normal position. The only indication as to the presence of the organ after being withdrawn into the body was the smear of the viscous material at the point where the organ entered the body wall. The time re-

quired for the deposition of a single egg from the time of its expulsion from the genital orifice until the egg was carried by the mouth-parts to beyond the margins of the body was nearly two minutes. This tick ceased oviposition due to the disturbance caused by the light of an electric desk lamp but resumed oviposition after the cover of the pill box container had been replaced as evidenced by the number of eggs which had appeared by the following day.

LIFE HISTORY STUDIES

Life history studies were conducted in the laboratory, and although the conditions best suited for the development of the various stages of ticks in the laboratory are to some extent unlike the conditions found in nature, the data obtained will give some idea as to what may be expected under field conditions.

A total of 168 larvae were used in this study representing the progeny of three female ticks, only the first batch of larvae produced by each female tick being used.

Incubation and Development of Ova

Oviposition by one female began April 8 and continued for a period of three days. At the end of this period the female tick was removed from the pill box and placed in a clean container for further observation.

The eggs were deposited in a loosely agglutinated mass easily separated by means of a dentist's probe and were of a light amber color when first deposited, darkening gradually in a few days. By the end of the 12th day the eggs darkened perceptibly on the upper side and the embryos were visible through the shell. On the 14th day the outlines of the six legs were visible and through the under surface of the eggs the outlines of the form of the developing larvae could be discerned. From that day until the eggs hatched (April 29 – completed May 1) the outlines of the body and legs were plainly visible, the eggs lost their luster and on the 21st day the larvae emerged. The shells split in a plane at right angles to the longitudinal axes of the bodies of the larval ticks. The mouth-parts of each tick emerged first, followed by the first pair of legs. Attention was focused on the hatching of one egg. Muscular efforts of the body of the young tick split the shell further, the second and third pairs of legs were ultimately extricated and the larva crawled from the confines of the shell. The young larvae upon emergence were quite active but were unable to feed.

Eggs were produced in batches ranging from a few to 140 or more eggs per batch over a period of several months throughout most of the year under laboratory conditions. The incubation period averaged 17 (range 9-24) days. The percentage of hatch varied with the different batches of eggs produced by several female ticks. In some batches none was apparently fertile while in others produced by the same tick 95% hatched. The greatest percentage of hatch for any one batch was 98%.

Developmental Stages

A few of the larvae were selected and placed on a young white mouse. They were unable to feed. A few days later some of these larvae were observed to molt. The slide mounts of these skins revealed the presence of three pairs of legs and the larvae emerging from the skin still possessed the characteristic three pairs of legs. Evidently many of the larvae of *Ornithodoros hermsi* may undergo two molts while in the larval stage—the first molt occurring shortly after emergence. The first

larvae fed to repletion on white mice on May 10, eleven days after hatching. Other feedings occurred on May 13, 15, 16, 17, 19, 20, and 21. The average time for feeding was 15.7 (range 7 to 40) minutes.

Second Molt, First Nymphal Stage.—The first nymphs appeared on May 26, the last on June 14, averaging 14.2 days from the date of feeding as larvae until the date of molting as first stage nymphs. The resulting nymphs were placed individually on separate white mice and allowed to engorge. These ticks fed on an average of 4.2 (range 1 to 41) days after molting. The time required to reach engorgement averaged 20.6 (range 11 to 131) minutes.

Third Molt, Second Nymphal Stage.—The first of the second stage nymphs appeared June 13 and the last on July 27, an average of 14.1 days from time of feeding as first nymphs until molting as second stage nymphs. These ticks were placed on individual white mice and permitted to feed. This stage required an average of 32.3 (range 12 to 66) minutes to reach repletion after an average period of 15.9 (range 5 to 38) days from time of molting to time of feeding.

Fourth Molt, Third Nymphal Stage and Adults.—Molting began on July 4, 15 days after the ticks had fed as second stage nymphs, the average for the entire lot being 17 days. With this molt, 10 ticks reached maturity, four females and six males. Feeding of the ticks was conducted in the same manner as before, the average being 29 (range 6 to 49) days after molting. Repletion was reached in an average time of 27.7 (range 10 to 70) minutes.

Fifth Molt, Adults.—With the fifth molt the majority of the ticks reached sexual maturity. Molting began August 12 and continued over a period of nearly two months, the last tick molting on October 10. As the ticks reached maturity they were paired (male and female). Eggs from these matings appeared August 24 with one female (Lab. No. 7/5) producing a first batch of eggs totaling 58. The egg to egg cycle thus covered a period from April 8 until August 24—4 months and 16 days, although egg depositions from other female ticks occurred at intervals from August to January.

OBSERVATIONS ON THE PROCESS OF MOLTING

Molting of the first nymphal stage was noted. The nymph, having fed previously on a white mouse, began the process of molting 15 days after feeding. The color of the young tick had darkened from a bright red to a dull, purplish-red shade with a greyish appearance over all. The claws of each leg of the tick were attached to the floor of the pill box in which the tick was confined. The first pair of legs spread wide apart in front of the body, the hind pair of legs held in a position under the posterior end with the two intermediate pairs of legs in a normal position along the sides. The first movements observed consisted of an upward tugging motion within the old loosened skin in an apparent effort to withdraw the legs. This was followed by a fairly rapid alternate contraction and expansion of the body. The fluid in the digestive diverticulae seemed to flow towards the central portion of the digestive tract and through muscular force exerted by the muscles of the body and digestive tract, the fluid surged out to the ends of the diverticulae in wave-like motions. This extra pressure caused the old skin to rupture at the anterior-most point, and as the body protruded forward the old skin split gradually following the lateral body margins until it had reached a point approximating the first coxa on

either side. As the body protruded still further through this opening, the mouth-parts were withdrawn from the old skin. Next the first pair of legs were withdrawn by an upward tugging motion of the body and with every tugging effort the legs were drawn towards the body from the old skin until each was free. All the while a continuous surging of the fluids in the body cavity was observed. In a short time the second pair of legs appeared and the tick was then able to fasten its claws quite firmly into the bottom of the pill box. The third and fourth pairs of legs appeared in succession, the tick resting between each exertion. The tick then crawled from the remaining cast skin. The entire process required, in this instance, approximately four minutes.

In some instances the cast skin of the tick was carried about on the posterior portion of the body until the next feeding at which time the old skin slipped from the distended body of the tick. Occasionally the legs of the tick became entangled in some manner, and the tick was unable to extricate itself from the skin, in which cases when noticed the skin was removed by means of a fine scalpel or scissors. The color of the freshly molted tick varied to some extent but the majority were of a deep garnet color with a clean, glistening, greyish sheen over the body, the legs being nearly white in contrast.

Occasionally, if temperature and humidity conditions were not correct, the ticks, particularly those of the first nymphal stage, were imperfectly developed, the legs being very papery in appearance and not rounded out. This first nymphal period is evidently the most delicate period in the development of the tick, more so than any succeeding molt.

NOTES ON LONGEVITY

In experiments dealing with longevity the ticks were kept in pill boxes in the constant temperature chamber in the laboratory and the results noted do not therefore necessarily represent the exact behavior of the ticks in nature.

Table 2 indicates the length of life of 3 adult female ticks (2 of which were

TABLE 2

Female tick number	Date taken as adult	Number of feedings	Number of eggs deposited	Number of infections in laboratory animals	Date of death	Elapsed time between date of capture and date of death		
						Years	Months	Days
10	9–19–33	3	50	0	3–15–35	1	6	–
5	8–17–34	4	232	4	10–21–35	1	2	4
7[1]	8–17–34	2	235	2	2–24–36	1	6	7

infective) taken as adults at Big Bear Lake, California. These ticks were permitted to feed several times during the interim from date of collection to date of death and during this period produced several infections in laboratory animals. The age, and the number of times these individual ticks had fed, previous to arrival in the laboratory is unknown.

One female tick (No. 11) was taken as an adult at Big Bear Lake, San Bernardino County, California, on August 17, 1934. This tick was kept under laboratory conditions for a period of 7 years, 3 months and 4 days. Assuming the life cycle to cover approximately $4\frac{1}{2}$ months, this tick would be over $7\frac{1}{2}$ years old from time of hatching until date of death.

TABLE 3.—*Showing longevity of female ticks from the time of hatching to May 15, 1942 and their ability to withstand starvation. (Ticks reared under laboratory conditions)*

Tick No.	Date hatched	Date last feeding	Elapsed time between last feeding and 5–15–42			Elapsed time between hatching and 5–15–42		
			Years	Months	Days	Years	Months	Days
1	5–20–35	11–23–37	4	5	22	6	11	25
2	6–14–35	11–29–40	2	5	16	6	11	1
3	"	5–25–38	3	11	20	6	11	1
4	"	5–23–38	3	11	22	6	11	1
5	"	11–24–37	4	5	21	6	11	1
6	"	5–26–38	3	11	19	6	11	1
7	"	11–24–37	4	5	21	6	11	1
8	"	6– 1–38	3	11	15	6	11	1

That ticks are able to withstand long periods of starvation is shown in Table 3. It is interesting to note that these ticks at present appear to be in a semi-dormant condition, that is, movements are sluggish and they refuse to feed when placed on a laboratory animal. The bodies are still rounded out from their last meal and egg deposition has ceased.

LONGEVITY OF NYMPHAL STAGES[1]

Third nymphal stage.—This series included three lots of third-stage nymphs—the progeny of three female ticks. The range of longevity was from one day to 108 days.

Second nymphal stage.—Records concerning the longevity of the second-stage nymphs show a range of from 25 days to 79 days from date of molting to death.

First nymphal stage.—A large series of ticks of this instar withstood periods of starvation ranging from 2 to 154 days.

LONGEVITY OF LARVAE

Two lots of larvae numbering well over one hundred lived without food from 30 days to 125 days.

EFFECTS OF THE BITE OF *Ornithodoros hermsi*

The generally severe bites of other species of argasine ticks have been described by many investigators. Nuttall et al (1908) cite instances in which the effects of the bites of *Argas persicus* were fatal to animals and to man on rare occasions. Livingstone (1858), referring to the wounds inflicted by the "tampan," *Ornithodoros moubata*, states the tick produces a tingling sensation which lasts for nearly one week. Dutton and Todd (1905) observed the bites of this species of tick to be quite painful and when allowed to bite a monkey noticed that, "Immediately after feeding a small crust of serosanguinolent fluid forms at the site of the bite. Surrounding it is a roseola about 2 mm in width. Two hours later the central clot is surrounded by 2 concentric zones, each 2 mm in width; the first colourless the second ecchymotic. Six hours later the clot has become almost black, and is placed at the apex of a slight colourless wheal, bordered by an ecchymotic zone about 1.5 mm in width." Wellman (1905–07) claims the bites of the young ticks to be more painful than those of the adults. Deaths resulting from the bites of *Ornithodoros turicata*, have been known to occur, according to Duges (Nuttall et al, 1908). *Ornithodoros talaje* also produces severe itching and pain by its bites (Guerin-Meneville, 1849). Herms (1916, 1939) describes the severe effects produced by the bite of *Ornithodoros*

[1] Longevity records of larval and nymphal stages represent periods of starvation from date of molting to date of death.

coriaceus and states that the natives in certain parts of California fear the bites of this tick more than the wound inflicted by a rattlesnake. Lesions produced on the skin of the arm of man "showed a small dark purple ring surrounding a bright red spot, the point of attachment. The discoloration disappeared in a short time, but the arm was 'highly irritable for two or three days and at the point of attachment a minute clear scab formed.'" Nuttall et al (1908) state, "their bites were intolerably sharp and painful, and wounds bled a good deal—but notwithstanding, there has been intermittent irritation ever since."

The bites of *Ornithodoros hermsi* appear to be quite benign in that persons contracting the disease, relapsing fever, seldom report having been bitten by any tick or insect (Porter et al, 1932). Larval ticks placed upon the arm of the writer and an assistant, engorged to repletion and detached in approximately fifteen minutes with no evidence of pain either at the time of the insertion of the ticks' mouth parts or at any time during the period of feeding. At the point of entry of the mouthparts a small hemorrhagic area appeared which was scarcely visible and would have been overlooked had we not known that the ticks had attached. Larval ticks allowed to feed upon white mice produced a more severe reaction in most instances. The wound itself evidently was not painful to these animals although the hemorrhagic area on the skin was larger, measuring on an average a little over 1 mm in diameter, with a bloody exudate usually forming in the center. Occasionally this area became swollen and tinged a dark purplish color resembling the type of wound inflicted by the Pajaroello tick, *Ornithodoros coriaceus*. The lesions produced by the nymphal ticks are also apparently quite benign and cause no evident discomfort to the experimental animal. In the majority of the wounds inflicted by adult ticks on experimental monkeys a bright hemorrhagic area appeared approximately five minutes after the mouth-parts were inserted into the skin of the animals. These areas averaged 3 mm in diameter forming a rather serrate-edged wheal. In the center of each area a blood-tinged exudate appeared as soon as the tick had detached. For a few days following the feeding of six adult ticks on a monkey, the animal was noticed to scratch vigorously at the wounds caused by the ticks. Results of experiments in which infective ticks were allowed to engorge on blood of humans demonstrated that the bite of the tick, *Ornithodoros hermsi,* produces little discomfort at the time of feeding. With but few exceptions no sensation was experienced by these volunteers during the time the ticks were attached. A slight stinging sensation was noted by two of the men at the time the ticks inserted their mouth-parts into the skin (Wheeler, 1938).

SUMMARY

Observations relating to the field biology of *Ornithodoros hermsi,* Wheeler, Herms and Meyer were based on notes taken by the author while in the field and supplemented in part by records from other investigators. Notes concerning life history studies were recorded as the ticks developed under laboratory conditions and therefore do not necessarily represent the true picture of the requirements for development in nature. From both field and laboratory observations:[2]

1. *Ornithodoros hermsi* occurs in the western United States at elevations above 3000 feet, with records of capture from California, Idaho, and Colorado.

[2] Observations on life history studies are represented by the author's own findings which may differ to some extent from those of other investigators.

2. This species of tick is apparently a parasite of small mammals, particularly of species of western chipmunks belonging to the genus *Eutamias*.

3. The act of oviposition is similar to that observed in other tick species. Eggs are produced in batches ranging from a few to 140 or more per batch. The greatest number of batches for any one female tick was four with a total of 232 eggs.

4. The incubation period of the eggs was 17 (range 9–24) days, while the percentage of hatch ranged from 0 to 98%. Ticks were reared in a constant temperature and humidity cabinet with optimum temperature and humidity of 75° F and 90 per cent respectively.

5. Development from egg to egg covered a period of four months and 16 days requiring five instars from egg to adult with many of the larvae passing through two instars. The process of molting is described.

6. Feeding of the ticks in various instars was accomplished by allowing the ticks to engorge on the blood of white mice immobilized in a cylindrical wire-cloth cage. The range of the average feeding times for the different instars was from 16 to 28 minutes.

7. Longevity of laboratory-reared ticks, with occasional feedings, was noted to cover a period of over six and one-half years from date of hatching to date of death. Infective adult ticks of unknown age taken in nature were observed to live in the laboratory for over one and a half years.

8. Female ticks were shown to be able to withstand starvation for over four years.

9. As compared to other species of *Ornithodoros,* the bite of *Ornithodoros hermsi* is quite benign as shown by experiments in which ticks were allowed to feed upon laboratory animals and human volunteers.

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CHARACTERISTICS OF THE POPULATION AVAILABLE FOR BIOASSAY OF ANTHELMINTICS IN *NIPPOSTRONGYLUS MURIS* INFECTION IN ALBINO RATS[1]

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The growing need for a more accurate method of assaying anthelmintics has suggested the use of *Nippostrongylus muris* infection in standard albino rats as a source of test material. Although it has been used in many of the recent quantitative experiments in immunity and resistance and once in analyzing anthelmintics (Schwartz and Porter, 1937), no experiments designed to discover how the worms varied from one host to another have been reported. Since such knowledge is a prerequisite to accurate statistical analysis of the proposed type of anthelmintic tests, the present experiments are reported to fill this lacuna and to suggest methods of stabilizing the technic.

MATERIALS AND METHODS

Albino rats from a commercial source or from our own stock, which is closely related, were used in these experiments. One group (#9) was made up of females; the rest were all males.

Dr. Asa C. Chandler of Rice Institute kindly supplied us with a culture of *N. muris* which he had used for a number of years and suggested methods for culturing the extra-host forms of the nema. These consisted of mixing fecal matter with helminthologically sterile animal charcoal and dirt, adding sufficient water to form a thick paste. This was placed on fluted filter paper in a Petri dish, leaving about one-quarter to one-half inch of the paper free of the culture. As the infective larvae develop they migrate to the free edges of the filter paper, from which they may be recovered by placing the feces-free pieces of filter paper in a tea strainer in a finger bowl full of 0.5 per cent sodium chloride solution. After standing for approximately one hour, the nemas were concentrated by centrifuging and draining the supernatant fluid so that 0.3 to 0.5 ml contained approximately 500 worms. Whenever samples for injection or counting were drawn from the suspension, it was always thoroughly agitated. In addition the suspension within the inoculating syringe was kept agitated with a small air bubble.

The actual number of larvae in the concentrated suspension was estimated by counting the worms in five 0.02 ml samples from a single tuberculin syringeful, and the amount of suspension necessary to infect each rat with approximately 500 worms was injected subcutaneously into the abdomen immediately medial to the anterior portion of the right fold of the flank. Since Chandler (1935) has shown that immune rats often react to subsequent injection by the formation of abscesses, this technic prevented the accidental use of immune animals.

The rats were killed on the 10th day with ether and the small intestine removed.

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The contents were first gently squeezed out with the fingers or a five-inch hemostat, then more vigorously with a round instrument and a glass plate. From time to time the stripped intestines were examined for worms, but none were ever found.

The intestinal contents were mixed with sufficient 5 per cent $NaHCO_3$ solution to fill a small (50 ml) bottle and allowed to stand in an ice-box for at least 24 hours. The bicarbonate solution cleared and dissolved the mucus and mucosa and unless the animal had access to food in the 24 hours prior to death, the only solid opaque particles left after the refrigeration period were the reddish worms.

The worms were counted by the following technic.

1. The supernatant fluid was poured into a filter paper in a funnel.
2. The remaining fluid containing worms and debris was vigorously shaken and poured into one or more cup-shaped filter papers on an absorbent pad.
3. The bottle was washed and the washings placed in the filter paper of step 1.
4. When the excess fluid was removed from the filter papers the worms were counted.

EXPERIMENTAL RESULTS

A. The Worms Injected

The characteristics of the worm suspension used for infecting the rats have been examined. To obtain material for statistical analysis five 0.02-ml samples were taken from each of 26 tuberculin syringefuls of a single suspension of infective larvae and counted, observing the usual precautions to keep the suspension uniform.

The sampling of the suspension of larvae was examined first by the analysis of variance in Table 1. The variation between syringefuls did not exceed the combined error between the five 0.02-ml samples from each of the 26 loadings to a significant degree, which indicated that the successive syringefuls were almost equally good samples of a uniform suspension. The variation between syringefuls in Table 1

TABLE 1.—*Analysis of variance of sampling of suspension of infective larvae of* Nippostrongylus muris

	Degrees of freedom	Sum of squares	Mean square
Between syringefuls	25	907	36.28
Within syringefuls	104	2904	27.92
Total	129	3811	29.54
		F = 1.29	

included two components, the variance associated with different fillings of the syringe and that between samples within fillings. When these were separated as suggested by Snedecor (1940) the first component was equal to 1.27 as compared with the much larger 27.92 within syringefuls. Hence the accuracy of estimates of the number of worms in a suspension could be increased nearly as well by increasing the size or number of counts from each loading of the syringe as from increasing the number of syringefuls. Neglecting differences between and within syringefuls the variance for a sample of 0.02 ml from this suspension was computed from the total row of the table as 29.54 and its standard deviation as 5.43.

The relative uniformity of the separate syringefuls suggests that the successive 0.02-ml samples from a single loading may have agreed with one another within the sampling error. In this case they should be distributed in the Poisson series, which

has the characteristic that its variance is equal to the mean. In the present case, the variance within syringefuls of 27.92 was considerably larger than the mean of 16.29 worms per 0.02-ml sample. Moreover the chi-square test for homogeneity of the 0.02-ml samples with their respective means for the separate charges of the syringe, computed as described by Fisher (1936), gave $\chi^2 = 174.39$ with $n = 104$. Since the variability exceeds that for random samples very significantly, it is clear that even the present precautions did not insure a uniform discharge of the larvae within the syringe in the five 0.02-ml samples. In practice approximately 500 worms or more than 30 such samples were injected at one time into the rats. For this reason, the lack of uniformity in the rate at which the larvae were discharged in small samples was smoothed out so that the variation in the number injected probably approached a more symmetrical and normal form. Yet the variability between loadings was even a little larger than that within syringefuls, emphasizing the need of testing the variability experimentally rather than estimating it on the basis of the Poisson series.

Keeping this limitation in mind, the combined variance obtained with the 0.02-ml samples ($s^2 = 29.54$) has been used to compute the error expected for a 0.5-ml sample. Since 0.5 ml contains twenty-five 0.02 ml, the observed variance for 0.02 ml was multiplied by 25 to obtain the variance of the total or $25 \times 29.54 = 738.5$, from which the standard deviation was estimated as the $\sqrt{738.5} = 27.18$ or 6.7 per cent of the 407 worms expected in a sample of this size. If only the errors of random sampling had been involved, the coefficient of variation would have been 5.0 per cent instead. This indicates that for accurate estimate of the number of worms in a suspension, the total volume examined should equal or exceed the quantity injected per rat. For the standard "dose" of 500 larvae in 0.5 ml, the error would then fall between 5 and 10 per cent of the estimate. Moreover, it would be preferable to sample several rather than one syringeful.

B. The Worms Established

Table 2 contains the pertinent information as to the age of rat, inoculating dose, numbers of worms establishing themselves, etc. Some of these rats had been treated by apparently ineffective anthelmintics. Analysis of variance of the number of worms established revealed a highly significant variation between tests. These variations were not due to any important difference between the variances of the separate groups, which when tested by chi-square did not differ significantly ($\chi^2 = 11.9$, $df = 10$).

The number of worms in the infected rats was subject to two unequal sources of variation; a large source between different groups of experimental animals and a smaller one within each sample group. In the absence of any seasonal or other type of curve to correct the secular variation, the ratio of the standard deviations in Table 2 provided the most satisfactory basis for adjusting the individual observations. Following the example provided by Bliss and Hansen (1939) a constant factor was added to each term in a group which would reduce the departure of the group mean from the general mean to $\frac{48.88}{248.80} = 0.1964$ of the original observed difference. This adjusted the individual values for secular variation so that the standard deviation based upon all 110 of the adjusted numbers of worms establishing themselves was the same as that observed originally within each sample population.

TABLE 2.—*Data and statistics of numbers of* Nippostrongylus muris *reaching maturity in groups of rats receiving the same dose of infective larvae*

Test number	1	2	3	4	5	6	7	8	9	10	11
Date of death of host	8/31/40	5/10/40	11/23/40	11/9/40	10/27/40	9/14/40	12/7/40	1/18/41	1/25/41	2/9/41	3/9/41
Age (days) of rats at time of infection	50	53	43	60	55	52	50	60	67	...	55
Av. wt. of rats at time of infection (grams)	140	140	125	134	135	132	111	136	112	149	127
Av. wt. of rats when killed (grams)	144	...	...	132	137	135	110	146	131	139	118
No. of larvae injected	520 ± 40	567 ± 70	505 ± 50	503 ± 40	504 ± 52	654 ± 153	493 ± 22	500 (?)	506 ± 17	508 ± 35	499 ± 60
No. of worms established (X)	113	362	219	292	314	129	349	338	378	172	318
	131	323	198	325	178	159	417	311	275	335	471
	121	357	197	303	315	130	299	274	412	335	346
	204	314	176	258	321	67	263	303	265	282	381
	212	352	286	243	295	129	244	279	286	250	340
	199	287	228	246	287	123	295	334	421	270	
	177	298	246	261	294	142	312	198	462	252	
	122	278	207	369	226	108	326	229	400	278	
		378	185	288	226	102	362	369	378	311	
		289	242	273	240	100	310	328	413	182	
		371	131	398				310		181	
										295	
Mean ($\bar{X}$)	159.9	328.1	210.4	296.0	269.6	117.0	317.7	297.5	369.0	261.9	371.2
Variance (V)	1,781	1,375	1,684	2,521	2,365	634	2,472	2,487	4,756	3,288	3,623
Standard deviation (s)	42.2	37.1	41.0	50.2	48.6	25.2	49.7	49.9	69.0	57.3	60.2
Coefficient of variation	26.4	11.3	19.5	16.9	18.0	21.5	15.6	16.8	18.7	21.9	16.2

Analysis of Variance of Data

	df	Sum of Squares	Mean Square	Standard Deviation
Between tests	10	619,051	61,905	248.80
Within test groups	99	236,480	2,389	48.88
Total	109	855,531		

F = 25.9

Since some of these corrections were positive and some negative, the general average number of worms establishing themselves in these rats remains the same. The resulting distribution was tested for both skewness and kurtosis (Snedecor, 1940). Neither g_1 measuring skewness nor g_2 measuring kurtosis exceeded its standard error ($g_1 = 0.132 \pm 0.230$ and $g_2 = 0.348 \pm 0.457$) indicating that the distribution was symmetrical and normal in form.

This distribution, however, was not binomial. Using the average number of worms established in these experiments from 500 infective larvae injected, a mean of 269 gave $p = 0.538$ and $q = 0.462$. According to the binomial theorem the variance pqn would be expected to equal $0.538 \times 0.462 \times 500 = 124.3$. The observed variance $\frac{S(x-\bar{x})^2}{n} = 2389$ or nearly twenty times the binomial value.

DISCUSSION

From the foregoing data it appears that the variation in numbers of *N. muris* infective larvae injected into rats under the conditions described was relatively small and probably normally distributed. Although the number of infective larvae in small-sized samples of the suspension varied rather widely, this irregularity was proportionately much smaller in the large samples injected into experimental rats under the conditions described.

However, an extremely large secular variation appeared in the number of adult parasites establishing themselves. This phenomenon has been noted by Chandler (1935) who was unable to find its cause. A part of it was undoubtedly due to the inaccurate estimate of the number of worms injected provided by the 0.02-ml samples.

The within-sample variation in numbers of worms established resembled that of the numbers of worms injected in that both were symmetrical and approached the normal curve in form. However, the former variation was relatively more extensive as measured by the larger coefficient of variation. This larger variation in numbers of worms established than in the numbers of worms injected predicates some action detrimental to the parasite on the part of the hosts used in these experiments, none of which were immune or age resistant.

Since the within-sample variation in *N. muris* infection was uniform and symmetrical even though large, it should be possible to determine with sufficient accuracy the numbers of these parasites killed by the lethal action of any drug from parallel counts of worms in treated and untreated infected rats. This fact has been used successfully to develop a new method of anthelmintic bioassay (Whitlock and Bliss, 1943) which is more accurate and less tedious than a Hall critical test with the present type of parasitism.

The use of a parasitic infection whose characteristics are known and can be controlled should be of as much assistance to the chemotherapy of enterohelminthiases as other standard infections are to the chemotherapy of syphilis, malaria, and various bacterial diseases.

SUMMARY

1. A standard technic for inoculating rats with uniform doses of *Nippostronglyus muris* is described and its error determined.

2. Analysis of data from 110 rats divided into eleven groups and infected with

uniform doses of infective larvae of *N. muris* revealed two types of variation in numbers of worms establishing themselves: a. A within-test variation which was larger than the binomial but uniform and approximating the normal curve; b. A between-test variation which was many times larger than the above and which was unpredictable.

3. The results of these experiments show that under adequate statistical and biological control it is possible to predict with workable accuracy the number of worms in rats of a treated group expected in the absence of treatment from the number of worms in a parallel control group.

4. Although part of the variation between and within separate tests was due to variation in the numbers of infective larvae injected into the rats, this did not explain the wide variation observed. More probably it was largely due to an interaction between host and parasite which has yet to be explained.

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A BIOASSAY TECHNIQUE FOR ANTHELMINTICS[1]

J. H. Whitlock[2] and C. I. Bliss[3]

The present paper describes a quantitative technique for the biological assay of anthelmintics in vivo which depends upon the mortality of a nematode in albino rats following graded doses of drug. Since small dead worms cannot be counted readily in fecal matter, the mortality of the parasite has been estimated from the numbers surviving in the treated animal and in parallel untreated controls. The practicability of basing mortalities upon the infection in the untreated host has been demonstrated by the study (8) of the numbers of *Nippostrongylus muris* which mature after the subcutaneous injection of approximately 500 infective larvae. Although the number varied considerably from one rat to another, the variation approached the normal curve in form. The limits were narrow enough to permit estimation of the mean number exposed to the drug in an experimental group of rats from the mean number of worms in a control group infected at the same time.

The percentage mortality of the parasite within the host has been related to the dose of drug so as to examine the frequency distribution of the just-toxic-dose. Earlier investigations on the toxicity of poisons to nemas in vitro (7, 9) have demonstrated the applicability of the normal distribution to the length of survival. If similar relations could be shown in vivo, the anthelmintic action of the drug could be defined more precisely than heretofore. By substituting exact definitions for the "minimal lethal dose" and similar concepts, it should be possible to replace terms such as

$$\text{chemotherapeutic index} = \frac{\text{minimal lethal or maximal tolerated dose}}{\text{minimal effective dose}}$$

with more reliable measures of therapeutic effect that are commensurate with the recent work in quantitative pharmacology. Current statistical procedures have been adapted to the present data as hereinafter described.

MATERIAL AND METHODS

The albino rats in these experiments were obtained from a single commercial source or from a closely related stock. At the time of testing they varied in age from 55 to 96 days, although within any one test group the range was three days or less. Using a technique that has been described elsewhere (8), they were infected with *Nippostrongylus muris* from a stock culture supplied originally by Dr. Asa Chandler of Rice Institute. Briefly, extra-host forms of the parasite from charcoal and earth cultures were suspended in a 0.5 per cent solution of sodium chloride and the number of larvae in suspension determined by a count of several random samples. An amount of suspension sufficient to infect each rat with a predetermined number of worms, usually 500, was then injected into the subcutis of the abdomen immediately medial to the anterior portion of the right fold of the flank. On the 8th day after

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the infection, the rats were treated with anthelmintic and on the 10th day killed and the worms recovered and counted.

As a result of preliminary experiments, rats were assigned at the time of treatment to the five or six groups in a test so as to balance body weight, except for those in Test III of the first experiment. Random assortment to groups, as used in Test III, perhaps, would have given equally good results. The balancing of body weights was intended to correct any possible bias in dosage, since all rats in a group received the same amount of drug. Within the weight range in these experiments 110–160 grams), however, no inverse correlation between efficiency and body weight has been noted.

The experiments fell into three main series and in all three series carbon tetrachloride served as the drug. The required dose was dissolved in mineral oil and administered through a metal stomach tube with a tuberculin syringe. In all cases the chemicals conformed to U. S. P. standards.

The first experiment was the most extensive. It measured the mortality of the parasite in the host at 4 to 5 dosage levels in five different tests. In Test I the four doses were varying amounts of a 10 per cent solution of carbon tetrachloride, but in the remaining series the dosages of drug were dissolved uniformly in sufficient mineral oil to make 0.5 cc.

The second experiment determined the dosage-mortality curve of carbon tetrachloride for albino rats. Although the rats were from the same source as those used in the first experiment, they were not infected with nematodes prior to treatment. As a second difference in procedure, the drug was administered to the rats in undiluted form without mineral oil or other purgative.

The third experiment tested the effect of increased "doses" of infective nematode larvae upon the efficacy of carbon tetrachloride. The standard inoculation of 500 larvae was doubled and doubled again to establish higher populations of parasites in the host. Eight days later each rat received a therapeutic dose of 0.26 cc of drug dissolved in sufficient mineral oil to make 0.5 cc and on the 10th day the surviving worms were counted in postmortem.

Statistical techniques are available (3) for the evaluation of dosage-mortality data, where both fatalities and survivors can be counted directly. Here, however, only the surviving nemas could be observed. The number of potential survivors killed by the anthelmintic had to be estimated by difference from the mean number of worms in the untreated control animals. Because the individual rats varied widely in their response, the usual computation has been modified, as will be noted later.

RESULTS OF EXPERIMENT I

The toxicity of carbon tetrachloride in solutions of mineral oil was determined in five tests involving 129 rats infected experimentally with *Nippostrongylus muris*. Table 1 shows for each rat the dose of drug administered and the number of worms recovered. The purpose of the experiment was to determine the relation between dosage of carbon tetrachloride and percentage mortality of the nematode. It was assumed in the computation that without drug treatment, each rat would have been infected with the same number of worms as the mean of the corresponding group of controls. The difference ($\times 100$) between the number expected and the number observed, divided by the number expected, gave the percentage of nemas in each rat killed by the drug.

TABLE 1.—*Number of adult* Nippostrongylus muris *recovered from albino rats ten days after injection with 500 larvae and two days after treatment with varying doses of carbon tetrachloride, with the mean and standard deviation for each group*

Test No.	Number of worms postmortem with following dose per rat of carbon tetrachloride				
I	Control	0.016 cc	0.032 cc	0.063 cc	0.126 cc
	279	328	229	210	63
	338	311	274	285	126
	334	369	310	117	70
	198				
	303				
Mean	290.4	336.0	271.0	204.0	86.3
S. D.	57.0	29.8	40.6	84.2	34.5
II	Control	0.032 cc	0.063 cc	0.126 cc	0.251 cc
	378	421	207	123	0
	275	462	17	143	0
	412	400	412	192	0
	265	378	74	40	25
	286	413	116	259	0
Mean	323.2	414.8	165.2	151.4	5.0
S. D.	67.0	31.0	154.4	81.4	11.2
III	Control	0.032 cc	0.063 cc	0.126 cc	0.261 cc
	172	252	273	194	10
	335	278	211	7	2
	335	311	294	222	0
	282	182	291	107	2
	250	181	231	161	1
	270	295	241	15	0
			298		
Mean	274.0	249.8	262.7	117.7	2.5
S. D.	60.8	56.4	34.8	91.1	3.8
IV	Control	0.08 cc	0.12 cc	0.14 cc	0.20 cc	0.26 cc
	381	349	309	180	3	1
	346	269	41	140	0	1
	340	314	372	22	84	0
	471	61	235	1	21	0
	318	468	299	275		
Mean	371.2	292.2	251.2	123.6	27.0	0.5
S. D.	60.2	148.8	127.1	113.7	39.1	.58
V	Control	0.10 cc	0.12 cc	0.16 cc	0.20 cc	0.26 cc
	297	128	317	5	3	0
	274	51	5	172	2	3
	300	176	65	132	0	0
	213	219	55	186	4	0
	199		116	11	5	
Mean	256.6	143.5	111.6	101.2	2.8	0.75
S. D.	47.5	72.0	121.4	87.4	1.9	1.5

The percentage mortalities were subject to variation (1) in the number of worms expected in the absence of the drug, and (2) in the susceptibility of the host to drug treatment. In an earlier study (8) with the same type of infection but without an anthelmintic, the number of worms recovered from each rat varied more than would be expected by chance. This has been checked in the present experiment. If the control rats in a given test had been equally subject to infection by nemas, the

TABLE 2.—*Analysis of the variance in the number of nemas which established themselves in the untreated control rats*

Variation	Degrees of freedom	Mean square	Variance ratio
Between tests	4	10468	3.011
Within tests	21	3477	1
Expected from binomial distribution		116	

variance observed between rats should approximate the theoretical sampling variance expected from the binomial distribution. The observed variances, however, were 18 to 39 times as large as those expected for equal exposure and equal susceptibility to infection, giving a combined standard deviation within tests of 59 worms. Despite this wide variation between individual rats, the average number of worms varied significantly between the five tests. Hence the number expected in each treated rat if the anthelmintic had been withheld could be estimated more accurately from the mean of the controls in the same test than from their general mean (302 nemas) over all five tests.

The above source of heterogeneity, however, accounted for only part of the variation between the rats in the treated groups. Both the observed variance and that expected from the binomial distribution were computed for all treated groups, omitting those where either the number of worms averaged more than in the controls or where one or more rats in the group were freed of them completely. The ratio of the observed variance to that expected by chance averaged 162.7 among treated groups as compared with 30.4 in the untreated controls. This five-fold increase in the variance was due, presumably, not only to the use of an "expected" instead of an observed value for the number subject to removal by treatment, but also to a significantly better response to the drug in some rats than in others.

THE DOSAGE-MORTALITY CURVE FOR *Nippostrongylus muris* IN VIVO

With many species of multicellular organisms, percentage mortality may be plotted against the dosage of drug as a sigmoid curve and the same relation would be expected here. Using methods described by Gaddum (6) and by Bliss (1), these curves can be transformed to straight lines and computed. With the equations for such lines one can determine the kill expected from a given dose of drug, or, conversely, the dose required to obtain a given mortality. Percentages are transformed by a table based upon the normal curve of error to units called "probits" (1), while doses are usually changed to logarithms. In the present case, however, the probits for the data in Table 1 could be plotted against dose directly as a single straight line rather than against its logarithm (Fig. 1).

For a precise determination of the dosage-mortality curve, the computation is made from "corrected" probits and weights (3). While corrected probits are essential for the observations at 0 and 100 per cent kill, they are also applicable to the intermediate mortalities. In terms of corrected probits, the mortality of the worms within groups of rats varied considerably less than in units of the empirical probits which are read directly from tables such as that given by Fisher and Yates (4). Hence, they have been used here in all computations and figures. In neither unit, however, was the variance related to the mortality in the theoretical form upon which the usual weighting coefficients are based. The variance within groups seemed to be either independent of the expected mortality or reduced at the larger doses. In consequence, the probit mortality determined from the worms in each rat has been given unit weight in computing the curves in Figs. 1 and 2 and in the analysis of variance in Table 3. The mortality from 0.063 cc of carbon tetrachloride in the second test averaged so much higher than would be expected from the remaining data as to suggest the presence of some extraneous factor. This mean has been identified by a shaded circle in the figures and omitted from the computations.

In contrast with the unstable mean susceptibility in many toxicological experiments, the individual dosage-mortality curves for the five tests of the present series agreed with each other well within the experimental error. The variation between

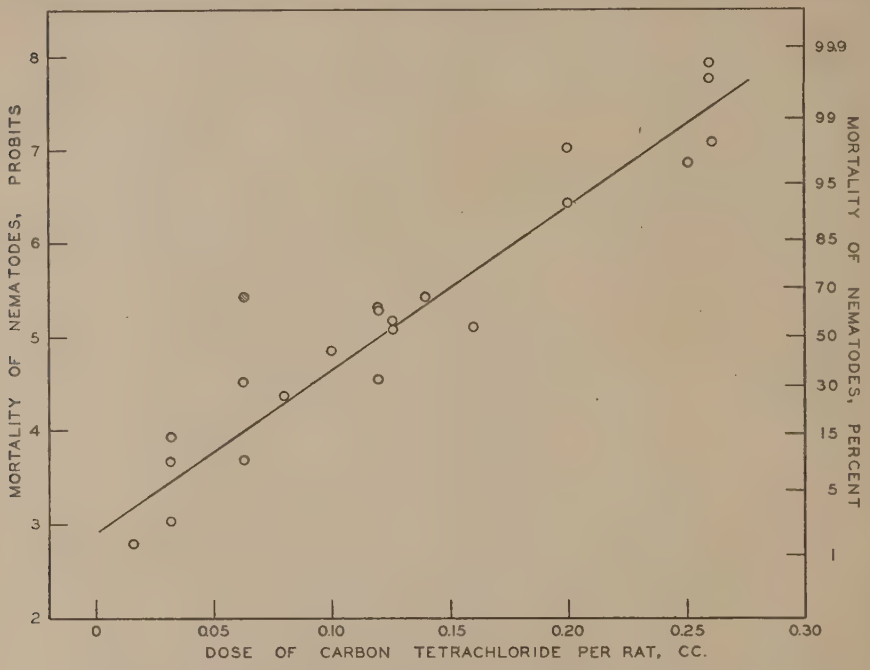

FIG. 1. Dosage-mortality curve for toxicity of carbon tetrachloride to *N. muris* in albino rats in terms of probits and dose, each circle representing the mean corrected probit for the kill of nemas in a group of three to seven rats. Shaded circle omitted in computing the curve.

the individual curves, both in position and in slope, has been compared in the analysis of variance in Table 3 with the scatter of the group means about the separate curves for each test and with the differences between rats within groups. It is evident

TABLE 3.—*Analysis of the variance between equally-weighted probits as a linear function of the dose of drug, omitting the observations at 0.063 cc in the second test*

Variation due to	D. F.	Mean square
Slope of single curve for all tests	1	181.421
Differences between curves in position	4	0.570
Differences between curves in slope	4	0.632
Scatter of group means about the curves for the five tests	11	0.748
Differences between rats within groups	79	0.997

from the analysis that the curves for the five tests did not differ from one another. The toxicity of carbon tetrachloride to *Nippostrongylus muris* in albino rats, therefore, seems to fall in the small group of cases having a dosage-mortality curve that is stable both in slope and in position.

The five tests in Table 1 could be represented by the single dosage-mortality curve plotted in Fig. 1 from the equation

$$y = 5.237\ [\pm 0.098] + 17.46\ [\pm 1.25]\ (x - 0.1334) = 2.909 + 17.46x,$$

where y is the mortality in probits and x the dose of drug in cc, and the square brackets [ ] enclose the standard errors for position and slope. According to this equation, no drug at all should kill 2.909 probits or 1.8 per cent of the worms. Yet more nematodes survived the smallest doses of drug in the first two tests than in the corresponding controls, which points to a threshold dose below which the drug is inactive or may even favor survival of the nematodes. Hence the dosage-mortality curve is not suitable for extrapolation to dosages below approximately 0.032 cc of drug, a range which fortunately is not of practical interest. The curve is exceptional in that probits have been plotted against the dose directly instead of against the logarithm of the dose so widespread in other toxicological studies.

The mortalities in probits were then replotted against the logarithm of the dose.

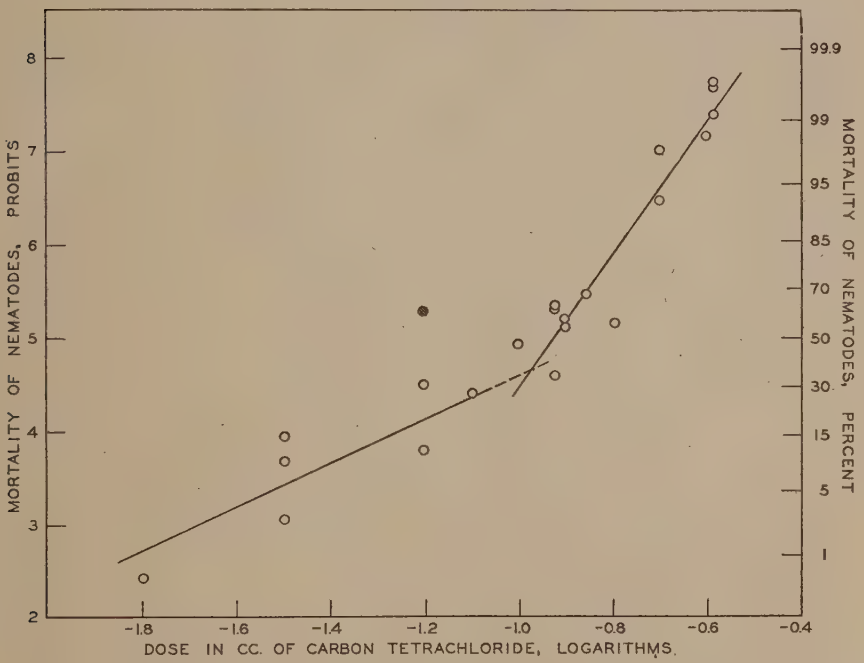

FIG. 2. Same basic data as Fig. 1 but with nema mortality plotted against the logarithm of the dose. Shaded value omitted in computing the curve.

At doses corresponding to 0.10 cc or more, the observations could be fitted by one straight line and at smaller dosages with a second straight line of lesser slope (Fig. 2). A change of slope in this direction at the lower dosages has been observed not infrequently in dosage-mortality curves, although it rarely disappears upon changing to an arithmetic scale as here. Some discontinuity between mortality and log-dose might be anticipated, indeed, from the heterogeneity between rats. A change in slope does not preclude computing a dosage-mortality curve that is valid for the upper range of mortalities. Since this is the zone of practical interest to the parasitologist so far as the parasites are concerned, the part of the curve below the "break" may be neglected. The equation of the curve plotted to the higher mortalities in Fig. 2 was

$$y = 5.999\ [\pm 0.099] + 7.144\ [\pm 0.683]\ (x + 0.7817) = 11.583 + 7.144x,$$

where y is the mortality in probits, x is the logarithm of cc of carbon tetrachloride, and the square brackets [ ] enclose the standard errors of position and slope.

For interpolation of the dose required for a given kill, the dosage-mortality curve in either Fig. 1 or 2 should indicate approximately the same value. For 99 per cent kill, for example, the expected dose from the curve in Fig. 1 was 0.253 ± 0.010 cc and that from the curve in Fig. 2, 0.254 ± 0.013 cc. Extrapolating, the dose expected to kill all but one worm in 500 as estimated from the first curve was 0.285 ± 0.012 cc and that from the second curve, 0.303 ± 0.020 cc. Hence, the logarithmic curve was the more conservative for extrapolation, even though not significantly different at 99.8 per cent kill from that based directly upon the cc of drug.

AN ALTERNATE TEST OF THE TOXICITY TO NEMATODES

Instead of expressing the effectiveness of the drug in terms of the percentage kill of nematodes within rats, one could measure the percentage of rats freed completely of nematodes at each dose. The first technique is the more efficient since a separate estimate of the mortality is available from each rat, while by the second procedure each rat is classified only as positive or negative. Moreover, even without an anthelmintic, the rats would eliminate most if not all of the nematodes if the examination were postponed for 10 days or two weeks. Hence, any apparent practical advantage in using the percentage of rats without nematodes is illusory.

For comparison with the former, preferred, technique, the percentage of rats freed completely of worms in each group of Table 1 has been changed to probits and plotted against the log-dose. Computed with corrected probits and weights, the equation for the dosage-mortality curve was determined as

$$y = 4.655\ [\pm 0.231] + 9.79\ [\pm 2.73]\ (x + 0.645) = 10.964 + 9.79x,$$

where y is the probit for the per cent of rats without worms, x is the log-dose of drug in cc per rat, and the square brackets [ ] enclose the standard errors for position and for slope. From this equation the dose which would be expected to eliminate the parasites completely from 99 per cent of the rats is the antilogarithm of $\bar{1}.628 \pm .080$ or 0.425 cc, and from 99.8 per cent of the rats $\bar{1}.685 \pm 0.095$ or 0.484 cc. In comparison with the percentage kill of nemas within rats, the doses were 2.4 times as large with an even greater discrepancy in the error of the estimate, so that the first technique used the host material much more efficiently. Whether it tends to underestimate the therapeutic dose cannot be judged from the present experiments, since all rats were examined at the same interval (2 days) after treatment.

TOXICITY OF CARBON TETRACHLORIDE TO RATS

The first experiment (Table 1) concerned the toxicity of carbon tetrachloride to the parasite in vivo; it was next essential to determine its toxicity to the host. The drug was administered to albino rats at four different dosage levels with the results shown in Table 4. The percentage kills have been transformed to probits and plotted

TABLE 4.—*Toxicity of carbon tetrachloride to albino rats*

Group No.	Dose of drug cc per rat	No. of rats	
		Treated	Dead
1	0.4	7	2
2	0.5	7	2
3	0.63	7	5
4	0.8	6	5

in Fig. 3 against the log-dose. The observations agreed satisfactorily with the straight line computed with both corrected probits and weighting coefficients ($\chi^2 = 0.818$, $n = 2$). In this case, too, the data would permit calculating the dosage-mortality curve directly from the dose with nearly as good a fit as for the logarithm of the dose. So many more cases have been reported, however, where the log-dose gives a straight line and the dose does not, that a normal distribution for the underlying susceptibilites in logarithmic units seems to be the rule and in arithmetic units the exception. Hence, when the data permit either interpretation as here, it is preferable to follow the rule rather than the exception.

The equation of the curve plotted in Fig. 3 may be written as

$$y = 5.040\ [\pm 0.259] + 5.76\ [\pm 2.48]\ (x + 0.259) = 6.533 + 5.76x,$$

where y is the mortality of albino rats in probits, x is the log-dose in cc per rat and the square brackets [ ] enclose the standard errors for position and for slope. The convex and concave curves in Fig. 3 indicate limits of one standard error below and

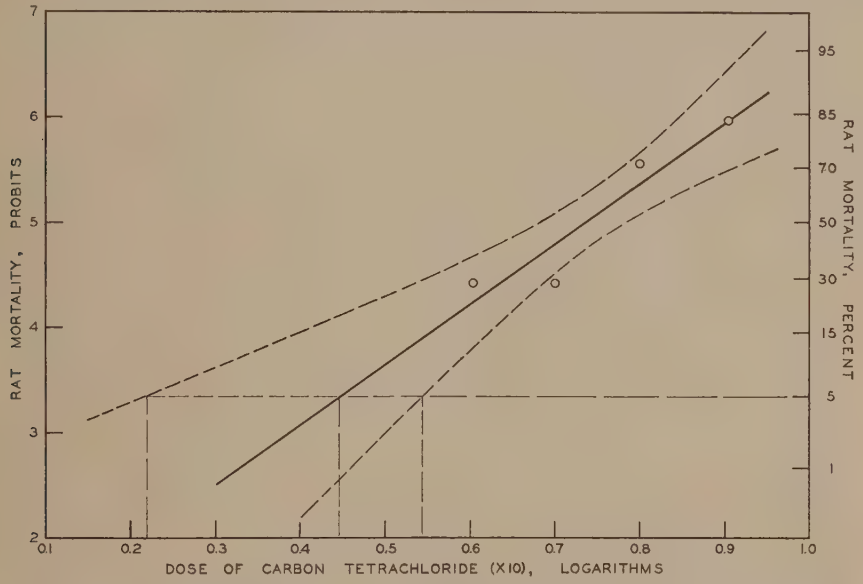

FIG. 3. Dosage-mortality curve for toxicity of carbon tetrachloride to rats. The broken lines fall 1 standard error above and below the curve. Their intersections with the ordinate for 5 per cent kill have been projected to the base to show the unequal errors above and below the dose expected to kill 5 per cent of the rats.

above the straight line. The greater the distance from the mean log-dose or mean probit, the more a point on the estimated curve is subject to the standard error of slope. Zones such as those enclosing the dosage-mortality curve in Fig. 3 apply equally to Figs. 1 and 2, but due to the larger number of determinations are there so much smaller that they have been omitted from the diagrams.

THE EVALUATION OF ANTHELMINTICS

The reliability of an anthelmintic increases with the ratio or difference between the therapeutic and the toxic dose. The so-called chemotherapeutic index is a loosely-

defined attempt to estimate this factor. With more complete knowledge of the relation between dose of drug and the biolytic reaction of the nematode and between dose and toxic symptoms in the infected host, the index can be evaluated much more accurately. Foster (5) has proposed that drugs be rated by a "standard safety margin," which he bases upon the ratio between the dose killing one per cent of the experimental animals (LD1) and that curing 99 per cent of them (ED99). He defines the standard safety margin as $\left(\frac{\text{LD1}}{\text{ED99}} - 1\right) 100$. When dosage-mortality curves are available as above, these doses and their ratio can be computed with a known accuracy. The "standard safety margin" has the added advantage of allowing for differences in the slope of the therapeutic and toxic curves and is not dependent upon the use of the same dosage unit (logarithmic or arithmetic) for both curves.

This procedure can be adapted readily to the evaluation of anthelmintics, using the mortality of the nemas in the rat as the measure of therapeutic effectiveness and mortality of the rat for the determination of toxicity. If the suceptibility of the rat to the drug is modified by the infection of worms, the toxicity should be determined with rats which have been inoculated in the same manner as those used in the therapeutic tests. The 99 and 1 per cent levels have the limitation that they are quite sensitive to errors in the slope of the dosage-mortality curve and hence are comparatively inefficient. They often represent extrapolations from the experimental data, and at its lower end the curve of toxicity may be modified by other factors as we have seen. It would be desirable, therefore, to alter the dosages to be compared from 1 and 99 per cent to 5 and 95 per cent respectively.

Sometimes drugs may be tested where the dose killing 5 per cent of the host is *smaller* than that killing 95 per cent of the parasite. In terms of the "safety margin" this would necessitate negative indices. The concept can be simplified, we believe, by using the ratio of the doses directly ($\times 100$) rather than the difference between the ratio and 1. We would propose defining the chemotherapeutic index as

$$\text{C.I.} = 100 \left(\frac{\text{LD5}}{\text{ED95}}\right) = \text{antilog } (2 + \log \text{LD5} - \log \text{ED95})$$

where LD5 is the lethal dose of drug killing 5 per cent of the host and ED95 is the "effective" dose killing 95 per cent of the parasites. A drug for veterinary use should have an index well over 100. From the equations for Figs. 2 and 3, the chemotherapeutic index for carbon tetrachloride has been determined as

$$\text{C.I.} = 100 \left(\frac{.2809}{.2036}\right) = 138 \text{ per cent.}$$

The proposed chemotherapeutic index is an estimate subject to the same errors as the two dosages from which it is computed. The resultant error of the index is complicated by unequal upper and lower limits in both numerator and denominator. In Fig. 3 the curve and its limits of error have been extended to intersect the probit corresponding to 5 per cent kill, indicated by a horizontal broken line. It is at once evident that in terms of the log-dose the lower limit is much further from the most probable value than the upper limit. This discrepancy is considerably less when the dosage-mortality curve is based upon more observations and when its mean mortality in probits approaches the value at which the dose is to be estimated, as in that for nematode mortality. Thus the LD5 for the toxic dose to the host of 0.281 cc

was determined within limits of 0.166 and 0.350 cc at odds of about 2 in 3, while the ED95 for the toxic dose to the parasite of 0.2036 cc fell between limits of 0.1964 and 0.2119 cc for the same odds.

Foster (5) has given a method for calculating the error of the standard safety margin from the upper and lower limits for both dosages. A simpler alternative based upon Eq. (28) in ref. (2) may be suggested for use where the dosage-mortality curves are computed in terms of probits and log-dose. Average variances for the log-LD5 and for log-ED95 are determined from the equation

$$V(X) = \frac{V(b)\,(Y - a)^2 + V(a)\,[b^2 - V(b)]}{[b^2 - V(b)]^2},$$

where V(a) and V(b) are the variances for position a $(=\bar{y})$ and for slope b respectively. The values computed from the dosage-mortality curve for the host are substituted in the above equation with $Y = 3.3551$ to obtain $V(X)_1$ and the equation is then solved again with the values from the dosage-mortality curve for the parasite and $Y = 6.6449$ to obtain $V(X)_2$. The upper and lower limits of the chemotherapeutic index (C.I.) for 1 standard error may then be calculated as

$$\text{C.I.} \overset{\times}{\div} \text{antilog} \sqrt{V(X)_1 + V(X)_2}.$$

Substituting the values from the present experiment, we find the limits were

$$138 \overset{\times}{\div} \text{antilog} \sqrt{0.026212 + 0.000272} = 138 \overset{\times}{\div} 1.454 = 200 \text{ and } 95 \text{ per cent.}$$

Although the toxicity of the drug to the parasite was determined within reasonably close limits, the relatively large error in the LD5 for the host limited the precision of the chemotherapeutic index. It is evident that for the best results, the dosage-mortality curve for the host should be comparable in accuracy to that for the parasite.

The dosage-mortality curves may be used for another, simpler comparison if so desired. Having determined the dose which will produce the desired kill of parasites, one may compute the expected mortality of the host at this dose. Thus, if 99 per cent kill of nematodes represents the therapeutic effect that is wanted, a dose of 0.254 cc of carbon tetrachloride is required. Substituting the logarithm of 0.254 or -0.595 in the equation for rat mortality, the expected mortality in probits is $y = 5.040 + 5.76(-0.595 + 0.259) = 3.105\,(\pm 0.871)$. This represents an expected mortality of 2.9 per cent of the rats, falling within limits of 0.3 and 15.3 per cent.

EFFECT OF NUMBER OF INFECTIVE LARVAE INJECTED

The relation between the number of parasites and the efficacy of the drug has been tested in a preliminary experiment. Ten rats were each injected with the standard number of 500 infective larvae, 4 with an average of 1060 larvae and 3 with approximately 2120 larvae per rat. Eight days later 5 animals in the first group and all of those in the last two groups were treated with 0.26 cc of carbon tetrachloride in mineral oil (q.s. 0.5 cc) which would be expected to kill over 99 per cent of the parasites. Although more mature nematodes were recovered without treatment than in the controls for the first experiment, no parasites survived in the rats receiving the standard number of larvae and only 4 parasites in one of the 4 rats receiving twice the standard number (Table 5). With a fourfold increase in infection only one parasite was recovered from one rat out of three. While the action of the drug at this dosage was relatively independent of the number of infective larvae, doses below the therapeutic level may well be more sensitive to the size of

TABLE 5.—*Relation of size of inoculation of* N. muris *to effectiveness of a dose of 0.26 cc of carbon tetrachloride diluted to 0.5 cc with mineral oil. Weights of groups when treated averaged from 113 to 155 grams*

	"Dose" of infective larvae			
	509 ± 82		1060 ± 171	2120 ± 341
	Controls	Treated	Treated	Treated
No. of worms postmortem	460 503 411 442 454	0 0 0 0 0	0 0 4 0	0 1 0
Mean	454	0	1	0.33

the infection. Hence, wide fluctuations in the number of larvae should be avoided in determining the toxicity of drugs in vivo to *Nippostrongylus muris*.

SUMMARY

Dosage-mortality curves have been determined experimentally for the toxicity of carbon tetrachloride to artificial inoculations of *Nippostrongylus muris* in albino rats. When percentage kills were transformed to probits, the observations could be plotted linearly against the dose or log-dose, a relation similar to that observed for anthelmintics on parasitic nemas in vitro and for lethal drugs on other muticellular organisms. Since the percentage mortality was estimated from parallel untreated control groups, the standard calculation required certain modifications which are described.

The toxicity of the drug to albino rats was determined with a similar dosage-mortality curve, so that the dose of carbon tetrachloride killing predetermined percentages of both parasite and host could be computed with a measurable precision. Based on these curves, two methods for evaluating anthelmintics are proposed, (1) a redefined exact chemotherapeutic index computed from the ED95 and the LD5 with a known error and (2) the expected host mortality corresponding to the required therapeutic effect.

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CAN LARVAE OF *COCHLIOMYIA AMERICANA* C. AND P. MATURE IN CARCASSES?

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Cochliomyia americana C. and P. is normally an obligatory parasite causing myiasis in mammals. In nature an infested animal if left untreated becomes subject to multiple reinfestations, which usually cause the death of the animal. Animals with simple infestations may die from other causes. It is important to know whether the immature larvae remaining in these dead animals can develop to maturity.

Experiments were conducted at Valdosta, Ga., in 1936–39 to determine what age a *Cochliomyia americana* larva must reach in a live animal in order to complete its development in a carcass.

EXPERIMENTAL METHODS

The technique used was simple. Animals were infested with newly hatched larvae and then sacrificed after various periods. In each test one or more animals were placed outdoors where they were exposed to carrion-feeding insects and usually at the same time at least one animal was placed in a screened room protected from attack. Each carcass was placed on a board on sand in a rectangular galvanized metal pan 6 inches deep. The outdoor pans were kept under sheds to protect them from rain. The sand in each pan was sifted daily, and the larvae were spread on a flat sheet of metal. All larvae of *Cochliomyia americana* were removed and placed in sand in jars. Emerging flies were placed in fly-rearing cages and tested for egg laying. Fifteen tests with larvae of 5 ages were conducted in a screened room and 24 tests with larvae of 8 ages were conducted outdoors.

RESULTS

The data accumulated are summarized in Table 1.

Under protected conditions *Cochliomyia americana* larvae develop readily in carcasses even when only 24 hours old at the death of the host animal. Younger larvae may continue their development, although in the few tests conducted no larvae 15 hours or 20 hours old matured. However, Melvin and Bushland* have been able to rear such larvae to maturity on an artificial medium composed of ground meat and blood. In fact, the present procedure in rearing stock in laboratories conducting research on screwworm problems is to feed the larvae on such medium.

Much more important is the fate of the larvae when the carcass is exposed to other carrion feeders such as occurs under natural conditions when animals die as the result of screwworm attack. Under such conditions larvae 24 hours old at the death of the host may be recovered, but they are not sufficiently mature to develop to the adult stage. Larvae 30 hours old were found in one test to mature, pupate, and emerge as flies, but in that test the blowfly population was exceptionally low. Larvae 48 hours old have a greater chance of maturing, whereas a high percentage

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* Melvin, Roy and R. C. Bushland 1936 A method of rearing *Cochliomyia americana* C. and P. on artificial media. U. S. Dept. Agric., Bur. Entom. and Plant Quar. ET–88. 2 pp. Mimeographed.

TABLE 1.—*Development of* Cochliomyia americana *larvae of various ages in carcasses screened and unscreened from attack by carrion-feeding insects*

Age of larvae at death of host: Hours	Number of tests	Average number of larvae placed in carcass (approximate)	Average number of larvae recovered	Degree of emergence and egg laying of recovered larvae
		Carcass screened		
15	3	300	0	.....
20	2	500	0	.....
24	5	340	95	Flies emerged and laid viable eggs in two out of four tests.
30	1	150	25	Flies deposited viable eggs.
48	4	237	181	High fly emergence and viable eggs always laid.
		Carcass unscreened		
24	6	400	9	No flies emerged.
24	6	400	0	.....
30	2	175	5.5	Flies emerged in one test and deposited eggs.
37	2	150	1	One fly emerged in one test but no eggs were deposited.
48	5	420	25.8	Flies emerged in three out of five tests and deposited viable eggs in two out of three tests.
54	1	500	329	Considerable emergence and many viable eggs deposited.
60	3	250	196	Considerable emergence; flies not tested for eggs.
76	4	Larvae recovered		Flies emerged, slightly subnormal in size but deposited viable eggs.

of those 54 hours old or older are fairly certain to mature and develop into flies that can deposit viable eggs.

There was no indication that the number of larvae per wound had any effect on the number of mature larvae recovered. It was found that larvae would continue to develop in carcasses even outdoors, but unless they were mature by the third day after death of the host they had little chance of continuing development, owing to competition with the many carrion-feeding larvae. Larvae 24 hours old and in the early second stage were observed to molt to the third stage by the third day after death of the host, but even if recovered these young third-stage larvae never developed to adults. Under outdoor conditions carrion-feeding larvae usually overran the carcass by the third day after death of the animal and *Cochliomyia americana* larvae could no longer be observed even though they had been seen as vigorously active 2 days after death of the host.

THE RELATIONSHIP BETWEEN THE INTESTINAL SIZE OF YOUNG MICE AND THEIR SUSCEPTIBILITY TO INFECTION WITH THE CESTODE, *HYMENOLEPIS NANA* VAR. *FRATERNA*

JOHN E. LARSH, JR.[1]

Several workers have noticed that very young mice are more resistant to infection with the cestode, *H. nana* var. *fraterna,* than are mice between two and three months of age. Woodland (1924) observed that the smallest and the largest mice are least liable to be infected naturally. Later, in controlled experiments, Shorb (1933) and Hunninen (1935) showed that considerably fewer worms develop in mice about one month old, than in those of the most susceptible age (about two and one-half months old). Although the differences are not as striking, the writer has recently noted this same relationship in a series of experiments involving young mice, most of which were 21 to 25 days old. The percentage development of cysticercoids in these mice was about six compared with about eight to ten in mice two and one-half months old. Of the suggestions offered to explain this difference, Hunninen's seems most plausible. In his opinion, the shortness of the intestine and the small size of the villi in the very young mice act as a mechanical disadvantage to the normal hatching and penetrating activities of the onchospheres. If this is really the reason for their greater resistance to infection with this tapeworm, any factor that would increase their intestinal size should make them more susceptible.

It has been firmly established that the anterior pituitary of higher animals has a special relation to bodily growth and splanchnomegaly. The only figures that were found which showed a specific action of this gland on the development of intestinal tissue are from experiments with juvenile pigeons (Schooley et al., 1937) in which the response was accredited to the hormone, prolactin. By repeated injections of this hormone after hypophysectomy, the body weight of the pigeons was increased by eight per cent, intestinal length by 15 per cent, "empty weight" (weight after contents are removed) of intestine by 10 per cent, and villus length by 17 per cent over the corresponding values found in unoperated controls. It seemed desirable to test the effect of this hormone on the development of intestinal tissue in young mice to determine whether a similar increase could be produced, and, if produced, whether it would increase their susceptibility to the tapeworm.

DESCRIPTION OF EXPERIMENTS

At various intervals, four separate experiments of the same kind were carried out. In each, there were three groups of mice. Two of the groups were about 25–26 days old at the beginning of the experiment and the other was about 65 days old. All the mice were checked at least three different times during the experiment by the Lane technique to make certain they were not previously infected. Beginning with the 26th day after birth, one group of the younger mice received a series of ten

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[1] From the Department of Helminthology of the School of Hygiene and Public Health of the Johns Hopkins University. For their helpful criticisms and suggestions made during the course of the work and in the preparation of the manuscript, the writer is indebted to Drs. W. W. Cort and G. F. Otto.

daily 0.1 cc injections of prolactin[2] which were administered subcutaneously over the pectoral muscles (Schooley et al., 1941). The other group of younger mice served as untreated controls. The third group of older mice was included as a control on the degree of resistance to infection of the younger mice. On the day following the last prolactin injection (when the younger groups were 37, the older one, 76 days old), all the mice were given by stomach tube a test dose of eggs of *H. nana* var. *fraterna*, which had been isolated from fecal pellets stored in tap water at room temperature for 48 to 72 hours. All the mice were sacrificed 93 hours after receiving this test infection, and the number of cysticercoids that had developed was used as an index of infection (Hunninen, 1935). Determinations were made on both experimental and control mice to find whether the prolactin had affected the intestinal size. With the intestine under approximately uniform tension, its length, from pylorus to cecum, was measured to the nearest centimeter on a meter stick. The "empty weight" was recorded after the mucus was removed—a procedure preparatory to cysticercoid enumeration. The length of the villi was measured from prepared microscopical sections of the duodenum 2–3 cm below the pylorus; measurements were taken in 10 different radial planes over at least 50 sections, and the average used.

As shown in the table, all four groups of the 41-day-old control mice had a considerably smaller percentage development of cysticercoids than did the older, 80-day-old controls, which agrees in general with the results of similar comparisons mentioned above.[3] On the other hand, three of the four groups of the younger mice that received prolactin treatment had percentages much higher than those of the untreated mice of the same age. In fact, these percentages were about the same as those of the second control group of 80-day-old mice. The other group that received prolactin (A of experiment 2), however, had a much smaller percentage development, which, in fact, was very much less than in any of the controls of the same age.

The response to prolactin treatment in all four experiments was striking. In the young mice treated in this way, there was an approximate 15 per cent gain in length of the intestine, a 54 per cent increase in "empty weight" of the intestine, and a 21 per cent increase in length of the intestinal villi over the corresponding values found in the untreated mice of the same age. In fact, the intestinal size (except for villus length) of the treated mice was almost the same as that of the mice in the older control group which were nearly twice their age.

DISCUSSION

The data given above indicate that prolactin affects very young mice in much the same way as young pigeons by increasing the length of the intestine, its "empty weight," and the size of the villi. This results in a much greater intestinal surface. Associated with this increased size of the intestine was an increased percentage development of the cestode, *H. nana* var. *fraterna*, in three of the four groups. In the other prolactin group (A of experiment 2) the resistance to infection was so much

[2] 100 prolactin units per cc prepared and standardized by the Armour Laboratories in accordance with the technique of Riddle et al (1933).

[3] It is interesting to note that the highest cysticercoid percentages (around 5) in mice two and one-half months old are only about half those recorded in the earlier study, although in both cases stored eggs were used for infections. The only explanation that can be offered for these differences is that the worms used in the two series of experiments were from entirely different sources.

TABLE 1.—*Showing the relationship between the increased intestinal size of young mice given prolactin and their susceptibility to infection with the cestode,* H. nana *var.* fraterna

Experiment No.	No. of mice	Range in length of intestine from pylorus to cecum (cm)	Range in "empty weight" of intestine from pylorus to cecum (gm)	Average length of villi (microns)	Egg dose	Total No. of cysticercoids	Range in No. of cysticercoids	Percentage development
A. Young mice (41 days old) treated with ten injections of prolactin								
1	8	38.5–45.0	0.55–0.94	430	1850	788	84–138	5.0
2*	8	43.2–50.1	0.72–0.95	438	1050	60	0–21	0.7
3	7	41.0–49.0	0.60–0.82	434	1670	589	78–92	5.0
4	7	43.0–49.0	0.60–0.85	427	1350	464	52–80	4.9
B. Young mice (41 days old) not treated with prolactin								
1	8	34.0–41.0	0.40–0.68	350	1850	416	39–63	2.8
2	8	38.7–44.2	0.31–0.69	360	1050	304	20–56	3.6
3	7	33.0–40.0	0.34–0.65	355	1670	356	15–70	3.0
4	7	33.0–40.0	0.36–0.49	365	1350	212	23–35	2.2
C. "Optimum" age mice (80 days old)—the most susceptible group								
1	8	35.0–47.1	0.67–0.78	495	1850	786	81–134	5.3
2	8	39.0–52.0	0.69–0.87	521	1050	428	48–82	5.0
3	7	45.0–51.0	0.68–0.84	502	1670	631	87–148	5.3
4	7	43.0–47.0	0.68–0.75	501	1350	529	69–102	5.5

* See discussion in text.

greater than in the controls of the same age that it seems probable it was due to an immunity transferred from the mother (Larsh, 1942). In spite of the fact that all mothers of mice used in these experiments were examined by the Lane method, there is no way of ruling out a previous infection lost before the experiment began. It is possible that there are other factors in this resistance which are affected by the prolactin, but the most reasonable explanation for the higher percentage development of cysticercoids in the other three prolactin groups is the increased length and much greater surface of their intestines over that in the untreated mice of this age. As Hunninen has pointed out, the ingested eggs of this tapeworm pass through the intestine at a rate which allows only a certain number to hatch, so that in very young mice with a shorter intestine, fewer cysticercoids might be able to hatch and penetrate the intestinal wall than in older animals. In addition, he thought that the smaller villus size in these mice might act as an obstacle to normal cysticercoid development. This was suggested because in older mice the cysticercoids are found in the body of the villi, while in the very young mice they are deep in the mucosa or at the base of the villi. The fact that in very young mice prolactin both increases the size of the intestine and reduces the resistance to infection with *H. nana* var. *fraterna* seems to support Hunninen's theory.

SUMMARY

It has been generally recognized that very young mice are more resistant to infection with the cestode, *Hymenolepis nana* var. *fraterna,* than are mice between two and three months of age. The suggestion has been made that this resistance may be the result of the shortness of the intestine and the small size of the villi which serve as a mechanical obstruction to the normal hatching and penetrating activities of the onchospheres. To test this hypothesis, young mice were given repeated injections of the hormone, prolactin, which has been shown to have a specific action on the development of intestinal tissue in young pigeons. Young mice that received these injections showed a striking increase in the size of the intestine over that found in controls of the same age. Associated with this was an increased percentage development of cysticercoids. It is suggested that this increased susceptibility of the very young mice that were given prolactin was due to the increase in intestinal size.

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STUDIES ON *PROTECHINOSTOMA MUCRONISERTULATUM*, N. G., N. N. (*PSILOSTOMUM REFLEXAE* FELDMAN, 1941), A TREMATODE (ECHINOSTOMIDAE) FROM THE SORA RAIL*

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In October, 1939, 12 of 39 *Stagnicola palustris elodes* (Say) which were collected in a marshy area near Black Creek, Wisconsin, were infected with a species of large echinostome cercaria. It was found that the metacercaria would develop in various species of pulmonate snails, and with further collections in the fall of 1940 and in 1941, the adult stage was cultured in young albino mice. In October, 1941, 6 adult worms were taken from the lower ileum of a field host, *Porzana carolina* (L.). With a description of those examples from the field host, the study was concluded and a manuscript was nearing completion when a paper by Feldman (1941) was published on the morphology and life cycle of the same species. Comparison of the two studies revealed minor points on which observations differed, and some additional data which Feldman's paper did not contain. The purpose of the present report is to record these supplementary data.

The cercaria which Feldman obtained from *Stagnicola reflexa* was declared to be identical with *Cercaria reflexae* Cort, 1914, and the adult which was obtained from experimentally infected chicks was thought to be a member of the genus *Psilostomum* Looss, 1898. Thus this species was introduced into the literature as *Psilostomum reflexae*. For several reasons to be discussed later, mainly because of its crown of spines, this species cannot be a member of the genus *Psilostomum*. It represents instead a new genus. Also, there is no justification for declaring the cercaria identical with *C. reflexae* Cort. It resembles *C. reflexae* but differs from it markedly, especially in size and spination. For *Psilostomum reflexae*, the new name *Protechinostoma mucronisertulatum*, n. g., n. n. is proposed.

THE LARVAL STAGES

The cercariae encyst in *Stagnicola palustris* and other species of pulmonate snails. *Lymnaea stagnalis*, *Physa* spp., *Helisoma trivolvis*, and *Fossaria* sp. as well as *S. palustris* all were infected in the laboratory, and individuals of each of these species were found to carry up to 40 metacercariae when collected from natural pools where cercariae were known to be present. The cercariae enter the snails through the respiratory orifice and encyst in the adjacent portions of the mantle, in the walls of the respiratory sac, or in masses that lie more or less free in the respiratory cavity. Attempts to infect fish and tadpoles were unsuccessful.

The descriptive data which follow on the larval stages include only those observations which either add to, or clarify the descriptions by Feldman.

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The Redia

(Figs. 3–4)

Mature stages very large, many over 2 mm in length. Each large example contains about 10 cercariae in various stages of development. Body almost unpigmented; the oldest ones sometimes bearing scattered minute dark orange colored granules. Collar regular and generally conspicuous in living specimens; not divided into distinct lobes. Birthpore close behind collar and often crowded into it. Pharynx relatively small, not capable of withdrawal behind collar. Flame cells in 2 groups on each side; one group at base of appendages, the other half-way between appendages and collar. Pulsating bladder on each side just anterior to appendages. Number of flame cells variable with size of redia; 9 in each group were observed in a specimen about 0.5 mm long, and in a well-extended specimen, 1.6 mm in length, there were 15 in each group. Immature cercariae were not observed free in the tissues of the snail. In larger hosts several thousands of rediae often were present.

The Cercaria

(Figs. 1–2)

Noteworthy features of the living cercaria are its relatively large size, slightly yellowish opaque cystogenous glands which fill most of the body, relatively small suckers, narrow excretory siphons containing numerous small granules, and large fins on both dorsal and ventral sides of the long and powerful tail. Fleshy collar inconspicuous but under favorable conditions, its crown of spines is easily observed. Pattern of collar spines identical with that of adult. Cuticular spines minute and evident only in preacetabular region. Cephalic ducts dorsal to oral sucker about 24 in number. Excretory bladder two-chambered; main excretory canals extend forward as enlarged siphons to anterior end and then extend unbranched back to extreme posterior end. Flame cells appear to be in groups of 3, with 12 anterior to bifurcation of esophagus, 6 in acetabular region, and 24 posterior to acetabulum on each side. Bifurcation of caudal excretory canal at junction of first and second fourths of tail. Dorsal fin low at base of tail, absent or extremely low along a short middle portion, and high along distal third. Ventral fin continuous from base to tip of tail but low near middle. Dimensions of average extended fixed specimens: body length, 0.50 mm; width at acetabulum, 0.16 mm; oral sucker, 56 by 50 μ; diameter of expanded acetabulum, 80 μ; contracted, 60 μ; pharynx, 30 by 24 μ; tail length, 0.67 mm; width of tail at base, 64 μ; greatest height of tail fin, 22 μ.

The Metacercaria

Cysts fairly uniform in size and shape. Even when crowded they are spherical, 0.252 to 0.280 mm in diameter. Cyst wall smooth and transparent, 17 to 20 μ in thickness. Body of metacercaria flexed ventrally and coiled. Growth rapid first week, slower but continuous for next 2 to 3 weeks. At 3 to 4 weeks, cuticular spination is well developed on all except postacetabular dorsal region of body; collar spines and anterior body spines of equal size, 4 μ long; gonads well developed, testes almost as large as oral sucker; oral sucker, 60 by 50 μ; diameter of acetabulum, 90 μ; pharynx, 40 by 34 μ; length of worm, about 0.6 mm; width at acetabulum, 0.22 mm; excretory system much advanced with lateral branching canals along siphons from excretory bladder to level of pharynx; all main excretory canals and bladder greatly dilated. No appreciable changes occur in the metacercariae after 4 weeks and they remain viable in the snails for at least 5 months.

THE ADULT

In agreement with Feldman's experience, it was found that the range of conditions under which *P. mucronisertulatum* will develop in laboratory hosts is very narrow. Feldman found that of several species of birds and mammals used, mature worms could be cultured in young chicks only, and even in these hosts the results were always uncertain. He also reported that metacercariae are infective after only 5 to 7 days of encystment, and that the adult worm is mature 5 days after infection. In view of the additional growth and maturation of metacercariae for at least 2 weeks after the first 5-day period, it seems doubtful if many are infective in so short a period.

The experiments summarized here were completed before adult worms were discovered in a field host. The field host (sora rail) being a bird, and Feldman's successful experiments being limited to young chicks, it is noteworthy that numerous

attempts to obtain infections in pigeons and chicks were unsuccessful while in 5 days after infection, albino mice produced mature worms about the same size as those from the bird hosts. Not all attempts to infect mice were successful, however, and feeding experiments with other mammals (5 albino rats, 5 hairless rats, 2 rabbits, 2 bats, and a guinea pig) were negative. Within a week after weaning, 10 mice were fed 100 to 200 metacercariae that ranged from 3 to 4 weeks of age. Five of the mice were found to contain no worms 2 days after feeding, and 1 was negative on the 5th day. From 3 of the remaining mice, 1, 1, and 14 worms respectively were recovered on the 2nd day after feeding. The 10th mouse was fed about 100 metacercariae on each of 4 consecutive days and was examined on the 5th day. Concentrated in the most distal 10 mm segment of the ileum, there were 72 worms of various stages of maturity, ranging from the very small 24-hour stage to mature worms with 1 or 2 eggs in the uterus.

In pigeons and young chicks the metacercarial cyst wall was undigested and the encysted metacercariae were invariably dead when they were passed in the feces. Apparently cysts sometimes were fractured, however, enabling the worm to escape. One living worm was found in the middle small intestine of a pigeon on the 2nd day after it had been fed metacercariae. In the feces of this pigeon there were 200 to 300 encysted metacercariae all of which were dead when passed. In bats most of the metacercariae excysted but were expelled in less than 3 days, and most of them were dead in the stools when passed. Metacercariae likewise excysted in rats but apparently did not live more than 1 or 2 days. The rats were fed 300 to 400 cysts each and were examined after 1 to 8 days with negative results in all but one instance. A black-eyed hairless, an albino hairless, and 2 common albinos were examined 24 hours after receiving metacercariae and while empty open cysts were found in each of them, only the black-eyed hairless contained living worms. In it there were over 300 worms, all in the lower ileum.

The passage of metacercarial cysts undigested through the entire digestive tract of some species of experimental hosts, notably the pigeon, while in others all of the metacercariae generally are liberated, suggests the absence of specific enzymes for digesting the cyst wall as an important factor in the limitation of hosts both in the laboratory and in the field.

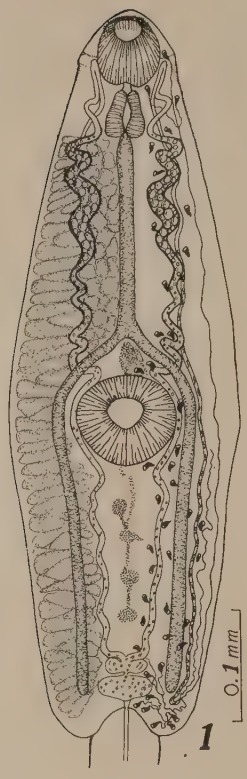
A pied-billed grebe (*Podilymbus podiceps*), 2 coots (*Fulica americana*), 6 muskrats (*Ondatra zibethica*), and 7 shrews (*Blarina brevicauda*), all known to inhabit and feed regularly in the immediate region where cercariae of *P. mucronisertulatum* were abundant, were uninfected with this species. The rail from which 6 specimens were taken was killed in the same watershed but about 10 miles distant from pools where cercariae were known to occur.

Studies on numerous specimens from experimentally infected young mice, 6 specimens from the rail, and 8 specimens which Feldman obtained from young chicks have brought to light several features of the adult stage that should be added to the earlier description.

Additional Data on Adult Morphology

(Figs. 5–10)

Cuticular spines extend almost to posterior end on ventral side but only to region of ovary on dorsal side of mature worms. Their arrangement is quincuncial and very regular. Fleshy crown weakly developed and not always evident on fixed specimens, especially when flattened. Crown spines barely if any larger than adjacent body spines. Typical number of crown spines is 51, with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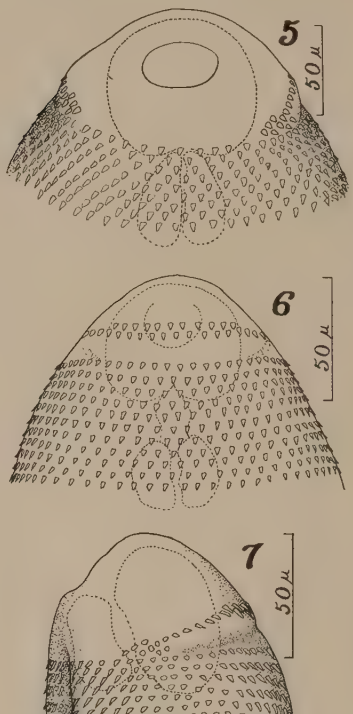
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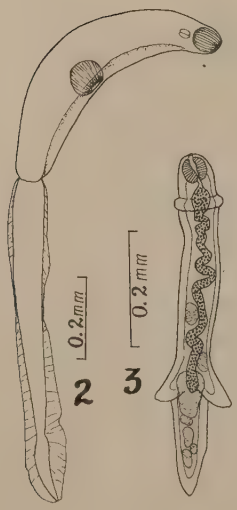
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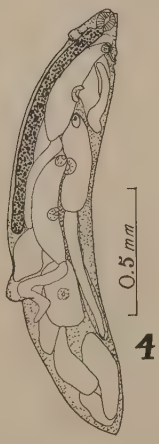
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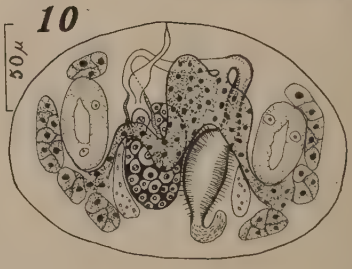
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very little variability in number and arrangement. Crown pattern: angle spines 7 in number, 3 anterior and 2 posterior ones in alternating rows that form a continuation of the lateral group, and 2 somewhat smaller ones in the angle between the body spines and the crown; lateral crown spines in a single row, 8 in number; dorsal group in 2 alternating rows, 11 in oral row, 10 in aboral; aboral row continuous with laterals. In young specimens just reaching maturity sperm ducts are dilated forming a vesicula seminalis externa on each side in region of acetabulum. Cirrus sac mostly anterior to acetabulum but may reach its posterior border. Seminal vesicle large and not divided into chambers; pars prostatica about half as long as cirrus sac; prostate gland cells occupy roughly half of cirrus sac. Cirrus unarmed. Laurer's canal to right of midline, short, without opening to outside. Seminal receptacle absent.

Protechinostoma n. g.

Diagnosis: Echinostomidae with small, linguiform, spinous body. Collar weakly developed, bearing a crown of spines barely if any larger than body spines. Crown spines arranged into angle, lateral, and dorsal groups as in *Echinostoma*, *Hypoderaeum*, and others. Angle and dorsal spines arranged in alternating pattern; the laterals in a single row. Acetabulum larger than oral sucker but relatively small for size of body. Cirrus sac anterior to the posterior border of acetabulum. Cirrus unarmed; prostate gland present; seminal vesicle single-chambered. Testes spherical to cuboid with smooth margins, situated about midway between acetabulum and posterior end. Ovary spherical with smooth margins. Uterus short, containing relatively few eggs. Vitellaria extend from posterior end to level of acetabulum. Seminal receptacle absent.

Type species: P. mucronisertulatum.

DISCUSSION

The new genus *Protechinostoma* stands in an intermediate position between the least specialized of the true echinostomes and the rather numerous echinostome-like genera (including some psilostomes) in which a distinct collar with a ventrally interrupted crown of spines is lacking. Of the true echinostomes it appears to stand nearest the genus *Hypoderaeum* Dietz, 1909 (1910). In both genera the crown spines are small, numerous, and somewhat similarly distributed on a weakly developed fleshy collar. In *Hypoderaeum*, however, the fleshy collar is more muscular, the acetabulum is large, the uterus is very long, and the testes are elongate as compared with *Protechinostoma*. In one species of *Hypoderaeum* (*H. mainpuria* Verma, 1935), the collar spines are so minute that they were overlooked entirely when the first description of it was made. The crown spines and the feeble muscular collar of *Protechinostoma* distinguish it from all echinostome-like genera in which either the spines or the crown is lacking. If in examining *Protechinostoma* spp. either or both of the crown structures were overlooked or misinterpreted, they might be confused with *Pseudechinostomum* Odhner, 1911, *Aequistoma* (Shchupakov,

Protechinostoma mucronisertulatum

Drawings made with the aid of a camera lucida.

FIG. 1. Cercaria. Ventral view of body, unflattened.

FIG. 2. Cercaria. Lateral view of extended fixed specimen.

FIG. 3. Immature redia. Ventral view.

FIG. 4. Mature redia. Lateral view, moderately contracted.

FIG. 5. Anterior end of adult. Ventral view of an unflattened fixed specimen from a rail.

FIG. 6. Anterior end of adult. Dorsal view of a small fixed specimen from a mouse. Glycerine mount under pressure.

FIG. 7. Anterior end of adult. Lateral view of mature fixed specimen from a mouse.

FIG. 8. Young adult. Ventral view showing characteristic dilated sperm ducts in a specimen from a mouse.

FIG. 9. Adult. Composite sagittal section through the genital pore, cirrus sac, ovary, egg, metraterm, and acetabulum.

FIG. 10. Adult. Composite transverse section through the ovary, Laurer's canal, vitelline reservoir, and oötype. Posterior view.

1936) Beaver, 1942, *Psilorchis* Thapar and Lal, 1935, or *Psilostomum* Looss, 1898. In *Pseudechinostomum* a well-defined crown is present but it is without spines, and differs otherwise from *Protechinostoma* by having a long uterus and a cirrus sac extending beyond the posterior border of the acetabulum. In the three other genera, crown structures are completely wanting. *Psilostomum* differs further from *Protechinostoma* in the shortness of its esophagus (Price, 1942). The crown structures of *Parorchis snipis* Lal, 1936, are like those of *Protechinostoma* but the two forms are otherwise dissimilar.

The resemblance between the larval stages of *P. mucronisertulatum* and *Cercaria reflexae* Cort, 1914, is at once apparent but does not reach the level of specific characters. *C. reflexae* is much the smaller and is without refractive granules in the excretory siphons. The most important difference between the two species, however, is the complete absence of crown spines in *C. reflexae*. Cort (1915) specifically points out that these structures are absent and there is no reason to suspect that they were overlooked. He noted that "The surface of the body to the region about half way from the acetabulum to the posterior end is covered with small spines arranged in rows and set closely together." If crown spines were present Cort would have seen them as easily as he saw the body spines. Sometimes allowances must be made for errors and omissions in different investigators' observations, but in this case the extreme allowances called for to bring *C. reflexae* into agreement with the cercaria of *P. mucronisertulatum* seem to be without justification.

SUMMARY

1. Additional data are reported on the cercaria, redia, metacercaria, and adult of *Psilostomum reflexae* Feldman, 1941.

2. It is found that this species is not a psilostome, but is instead an echinostome having a weakly developed crown. It represents a new genus for which the name *Protechinostoma* is proposed.

3. The cercaria which was originally reported as identical with *Cercaria reflexae* Cort, 1914, is shown to be a distinct species. A new name, *Protechinostoma mucronisertulatum*, is proposed for it.

4. A new laboratory host, the albino mouse, and a field host, the sora rail (*Porzana carolina*), are reported for the adult stage.

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THE LIFE HISTORY OF *LECITHASTER CONFUSUS* ODHNER (TREMATODA: HEMIURIDAE)[1]

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During the summer of 1941, we found a cystophorous cercaria developing in the marine snail, *Odostomia trifida*, collected from Eel Pond extension of Waquoit Bay, Massachusetts. Experimental determination of the life history of this cercaria has demonstrated that its adult stage is the hemiurid trematode, *Lecithaster confusus* Odhner, 1905, a widely distributed intestinal parasite of marine fishes. Copepods were found to serve as the second intermediate host.

A number of marine and freshwater cystophorous cercariae have been described, but very few of their life cycles are known. Those that have been traced experimentally are limited to species of *Halipegus*, a curious group of freshwater hemiurids which live in the oral cavity and eustachian tubes of amphibians. On the basis of morphological agreement between *Cercaria cystophora* Wagener and *Halipegus ovocaudatus*, Sinitsin (1911) states that Leuckart anticipated their genetic relationship but incorrectly assumed that frogs became infected directly, without the mediation of a second intermediate host. Sinitsin (1905) reported that metacercariae of *H. ovocaudatus* developed in nymphs of the dragon fly, *Calopteryx virgo*. Thomas (1934) described a cystophorous form, *Cercaria sphaerula*, which, when ingested by *Cyclops vulgaris*, developed as a metacercaria free in the body cavity of that copepod. Krull (1935) traced the life history of *H. occidualis*, for which he described a cystophorous cercaria and showed that dragon fly nymphs served as second intermediate hosts. In determining the life cycle of *H. eccentricus*, Thomas (1939) observed that the cercaria, a cystophorous larva, was ingested by *Cyclops vulgaris* and *Mesocyclops obsoletus* and that the metacercaria developed in the body cavity of these hosts. He reported that when infected copepods were eaten by tadpoles, the young worms remained in the stomach until metamorphosis, after which they migrated to the oral cavity and eustachian tubes where they occur in adult frogs.

Although the life histories of no marine hemiurids have hitherto been traced experimentally, their metacercariae are known to occur in a variety of marine invertebrates, chiefly the small crustaceans. Dollfus (1923) recorded them from the copepods, *Acartia clausi* and *Centropages hamatus* and later (1927) identified as a species of *Dinurus*, the progenetic metacercaria which Bonnier had found in *Ceratas-pis monstrosa*. Lebour (1923) reported the metacercaria of *Hemiurus communis* in the body cavity of a species of *Acartia*. Hemiurid metacercaria have also been observed in such marine animals as chaetognaths and ctenophores, which probably are accidental hosts not ordinarily included in the life cycle. These and other references to hemiurid metacercariae are given by Thomas (1934).

Lecithaster confusus, whose life history is the subject of the present study, has

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been reported from a number of marine fishes. Linton (1940) lists as hosts of this trematode in the Woods Hole region: *Centropristis striatus, Clupea harengus, Etrumeus sadina, Pomolobus mediocris, Poronotus triacanthus, Scomber scombrus, Spheroides maculatus,* and *Tautogolabrus adspersus.* To this host list of *L. confusus* may be added the killifish, *Fundulus heteroclitus* and *F. majalis,* and the four-spined stickleback, *Apeltes quadracus,* which we have found to be infected in nature.

MATERIALS AND METHODS

Since an unlimited supply of the various stages in the life cycle of *L. confusus* was available, living material was studied as much as time permitted. Permanently stained material was found useful, however, for certain observations and measurements.

For experimental infections, we used sticklebacks from the Eel Pond (in Woods Hole and not to be confused with a part of Waquoit Bay which is about ten miles from Woods Hole), and copepods of the genus *Acartia* from Vineyard Sound since stages of *L. confusus* were not found in any of a number of copepods and sticklebacks collected from these localities. The copepods were placed in finger bowls of sea water containing infected snails. Half of the bowls were covered with cloth and placed under a stream of filtered sea water while the others were left uncovered, but with a continuous flow of water on the outside of the bowls to maintain a favorable temperature. Although the copepods lived longer in the uncovered dishes, they did not survive beyond nine days. During that time, specimens were removed daily to observe growth and development of the metacercaria. The excretory pattern of the adult was investigated in both young and mature specimens. To observe the full length of the long excretory tubules, it was necessary to flatten the worms and manipulate the cover glass so that a semi-lateral view was obtained.

OBSERVATIONS

Proof of the Life History of *Lecithaster confusus*

While we were unable to use laboratory-reared experimental animals, it was found that copepods and fish, collected from the localities mentioned above, were free from infection with stages in the life history of *L. confusus.* When animals from those localities were exposed to infection in the laboratory, they were subsequently found to harbor stages of *L. confusus* which agreed in every respect with those found in naturally infected hosts from Waquoit Bay. Furthermore, the incidence and intensity of experimental infection were significantly higher than in animals collected from Waquoit Bay where *L. confusus* probably is the most abundant trematode parasite of fish. Thus, of several hundred *Acartia* collected from Vineyard Sound and exposed to cercariae of *L. confusus,* practically all became infected, some with as many as 15 metacercariae, while no worms were found in any of 55 control copepods. Only ten per cent of the copepods collected at Waquoit Bay harbored natural infections of *L. confusus* and few of those contained more than one metacercaria each. Similar results were obtained in experiments with the definitive hosts; adults of *L. confusus* were recovered from all of 25 sticklebacks, collected from the Eel Pond at Woods Hole and fed infected copepods while 40 control fish from the same place were negative for that parasite. Experimental fish were fed naturally infected *Acartia* because of the difficulty of keeping experimentally infected copepods alive.

However, experimentally infected *Acartia* were examined daily for nine days and development of the metacercariae was followed carefully to a stage at which they were identical to larvae recovered from naturally infected copepods. It therefore seems highly improbable that we have confused stages in the life history of more than one hemiurid species.

In an extensive survey of fish parasites at Waquoit Bay, we have found only two hemiurids, *L. confusus* and *Hemiurus appendiculatus*. The metacercaria of the latter also occurs in *Acartia* but is so strikingly similar to the adult, an appendiculate form, that it could hardly be mistaken for the larva of *L. confusus*. A cystophorous cercaria, which resembles *Cercaria sinitzini* Rothschild, has been collected only once from Waquoit Bay and has not been available in numbers sufficient for an experimental study of the life history. It probably is the larva of *H. appendiculatus*, which is much less common in Waquoit Bay copepods and fishes than is *L. confusus*.

Description of Stages in the Life History

The redia

(Fig. 14)

Only rediae producing cercaria were observed. They are non-pigmented, elongate larvae with somewhat tapered ends. The largest ones observed were 2.5 mm long and 0.08 mm wide. Mature rediae are constricted at intervals and contain most of the mature cercariae in the expanded regions between constrictions. The posterior end of such a redia is frequently shrunken and degenerate (Fig. 14) with its supply of germ cells apparently exhausted. Since no birth pore was observed, mature cercariae probably escape by rupture of the redial wall in the regions distended with fully developed larvae.

The cercaria

(Figs. 1–11)

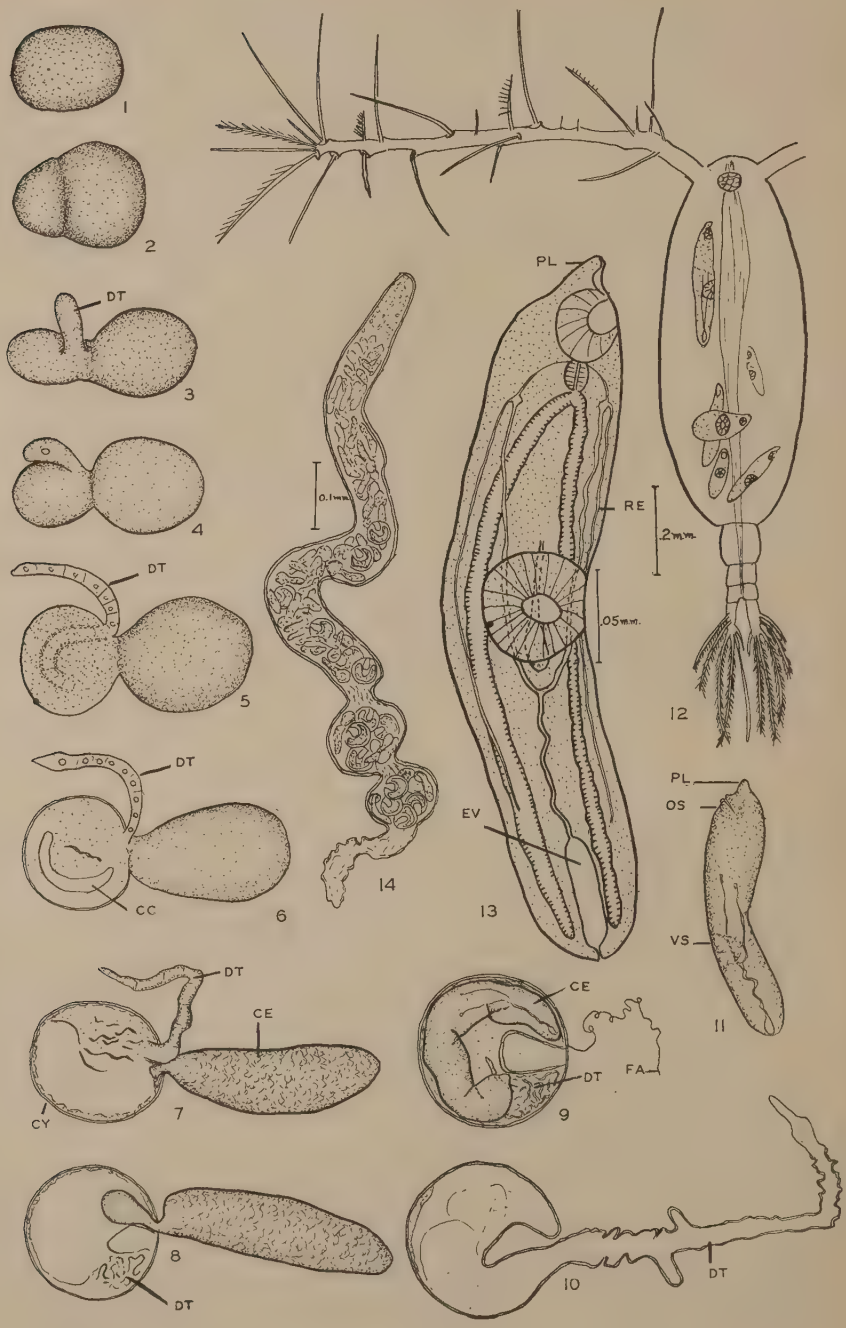
Specific diagnosis: Non-swimming cystophorous cercaria with discoid bulb or cyst 0.05 mm in diameter and having very delicate filament as only external appendage; delivery tube enclosed within cyst when cercaria emerges from snail; everted tube 0.15 mm long and 0.01 mm in diameter near cyst, distal half about 0.007 mm in diameter except slight enlargement near tip; everted tube with two short projections on opposite sides, one usually closer than the other to cyst. Cercarial body with smooth cuticle and short pre-oral lobe; average body length 0.085 mm, width 0.02 mm; structure poorly defined, even suckers being indistinct. Excretory vesicle a delicate Y-shaped tube with arms extending about half way to anterior end of body. Other structures indistinguishable in living cercariae.

Host: Odostomia trifida (Totten).

Locality: Eel Pond extension of Waquoit Bay, Massachusetts, U.S.A.

Fifty per cent of several hundred *O. trifida*, collected from Waquoit Bay, were found to be infected with the cercaria of *L. confusus*. The larvae escape in large numbers from snails isolated in the laboratory. Since they are extremely small and incapable of swimming, the cercariae are easily overlooked.

Observations on the embryology of the cercaria were made, following development from the germ ball to the stage which escapes from the snail. The germ ball is at first a subspherical mass of cells (Fig. 1) which soon becomes divided by a transverse constriction into two parts, the future bulb, cyst, or caudal vesicle, and the cercarial body. The delivery tube is first seen as a short outgrowth from the primordium of the bulb (Fig. 2). It gradually elongates (Figs. 3–6) and is seen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to consist of a column of cells. At this stage, a cavity appears in the cyst. With continued growth of the cyst, the delivery tube becomes hollow and is withdrawn into the bulb where it is seen as a closely coiled mass at one end of the cyst cavity (Fig. 8). Finally, the cercarial body withdraws into the cavity, the opening apparently becomes sealed, and the larva is fully formed. The delicate thread on the outside of the fully developed cercarial cyst probably is homologous with one of the various appendages described for other cystophorous cercariae; its origin was not determined in this study.

When the cercariae escape from the snail, they appear spherical but actually are slightly flattened. When mounted in sea water, light cover glass pressure causes excystment much as described by Willey (1930) for *Cercaria projecta.* In the process, the delivery tube is everted forcibly and the larva passes through it so rapidly that many observations were necessary to determine that the larva emerged at the tip of the tube rather than from one of the lateral projections. When the tube evaginates, the larva elongates as it shoots to the outside apparently as a result of intracystic pressure and elastic recoil of the tube. Passage of the larva through the delivery tube was observed by Willey (1930) for *Cercaria projecta,* Krull (1935) for *Halipegus occidualis,* and Rothschild (1938) for *Cercaria sinitzini.* It therefore seems likely that the process of excystment is much the same in all hemiurid cercariae although Thomas (1939) was unable to observe passage of the body through the delivery tube in the larva of *Halipegus eccentricus.*

While we have not observed excystment in the body of the copepod, the delivery tube probably pierces the intestine and the lateral extensions serve to anchor the tube in place until the larva passes through it into the body cavity of the host. This function of the delivery tube has been reported by Krull (1935) for the cercaria of *H. occidualis.* Due to the lack of penetration glands or even functional suckers in the cercaria, it is difficult to conceive any other means whereby it could reach the body cavity of the copepod.

The metacercaria

(Figs. 12 and 13)

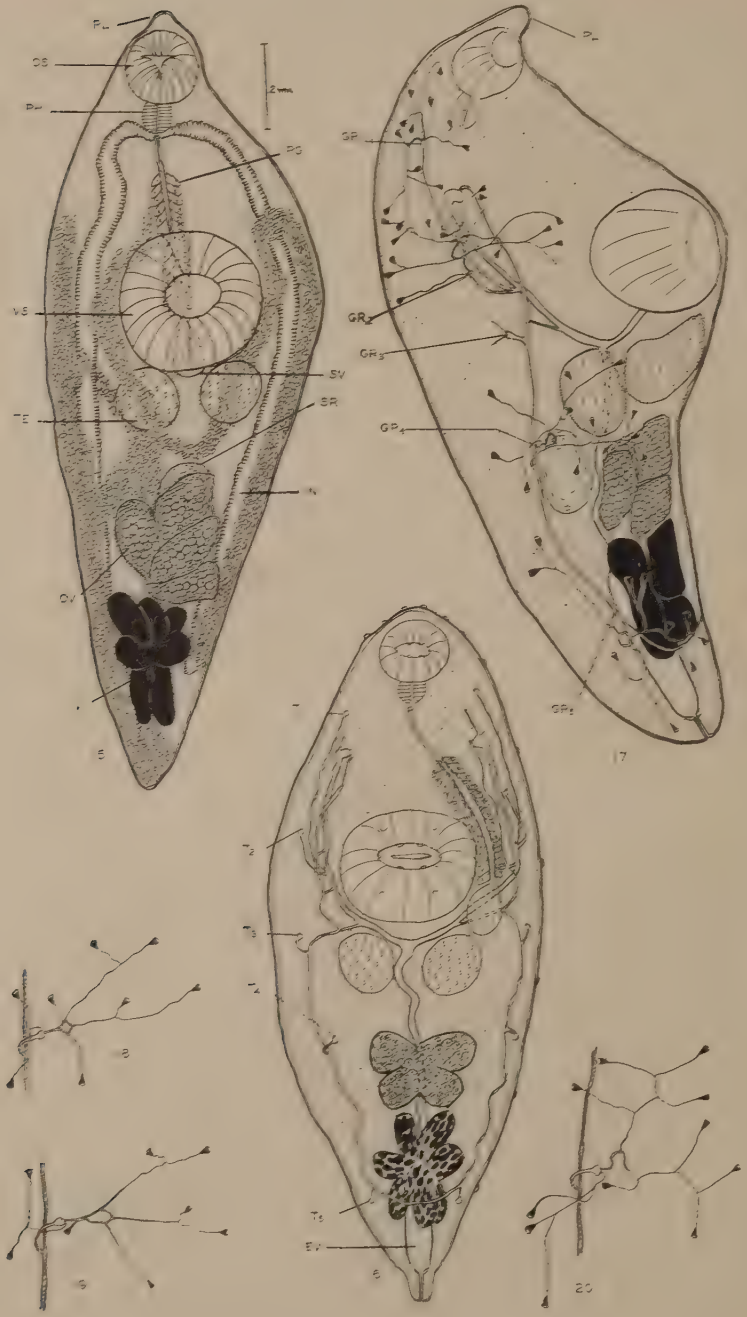
Data concerning experimental infection of copepods have been presented above as evidence contributing to the proof of the life history. The metacercariae of *L. confusus* occur unencysted (Fig. 12) in the hemocoele of *Acartia tonsa* where they are quite active and move about freely. The cuticle is aspinose and lacks the segmented appearance and caudal appendage of the larva of *Hemiuris appendiculatus* which also occurs in the body cavity of *Acartia.*

Growth and development of the metacercariae in *Acartia* are rapid. In nine days, the larva (Fig. 13) increases in length from 0.08 to almost 0.4 mm, suckers

Lecithaster confusus

FIGS. 1–9. Stages in development of cercaria from germ ball.
FIG. 10. Cyst showing everted delivery tube through which cercaria has emerged.
FIG. 11. Body of cercaria after escaping from cyst.
FIG. 12. Experimentally infected copepod containing six metacercaria.
FIG. 13. Nine-day metacercaria from *Acartia tonsa.*
FIG. 14. Redia.

CC—cyst cavity	DT—delivery tube	PL—preoral lobe
CE—body of cercaria	EV—excretory vesicle	RE—recurrent excretory tube
CY—cyst	FA—filamentous appendage	VS—ventral sucker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become well formed, and the excretory and reproductive systems attain a fairly advanced stage of development. Average measurements in millimeters of moderately extended, living, 9-day metacercariae are as follows: body length 0.39, width 0.094; width of oral sucker 0.06, of ventral sucker 0.075; pre-oral lobe 0.02 long; pharynx diameter 0.028. A prepharynx is lacking and the intestinal ceca extend to the posterior end of the body.

In 9-day metacercariae, the Y-shaped excretory vesicle consists of an expanded posterior sac with a slender median tube extending almost to the ventral sucker and dividing to form two arms which terminate anteriorly at the sides of the pharynx. A delicate transverse tubule, uniting the slightly expanded terminations of the arms, represents the anterior commissure characteristic of many adult hemiurids. In *L. confusus,* however, this connection disappears and cannot be detected in the adult worm. The presence of an anterior commissure in the metacercaria of this species supports Yamaguti's (1934) opinion that certain genera lacking the commissure, viz., *Lecithaster* and *Hysterolecithoides,* form an aberrant group of the family HEMIURIDAE.

The adult

(Figs. 15–20)

It is unnecessary to redescribe completely the adult of *L. confusus* since fairly complete accounts have been given by Odhner (1905), Looss (1908), and Linton (1940). These authors did not mention the small, contractile papillae occurring in the suckers and on the body; they are visible only in living material and even then may be contracted and consequently overlooked. Those on the body (Fig. 16) are dorsolateral in position and are seen clearly only in obliquely dorsal and ventral views of the worm. General morphological features of the adult *L. confusus* are shown in Figs. 15–17 which are self-explanatory.

DISCUSSION

Descriptions of the excretory systems of hemiurids are practically non-existent in the literature. Our observations on *L. confusus,* while incomplete, show that the system is extremely complex and apparently subject to considerable variability in the adult stage. Observations of the excretory pattern in both immature and mature adults indicate that some of the variations may be correlated with the size of the worm.

The excretory vesicle of the adult is much as described already for the metacercaria. A short canal leads from the vesicle to the pore which is situated at the posterior tip of the body. The expanded portion of the vesicle reaches almost to the anterior margin of the vitelline lobes. From that level, the vesicle continues as

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Lecithaster confusus

FIG. 15. Mature adult.
FIG. 16. Adult showing principle excretory tubules.
FIG. 17. Lateral view of adult showing excretory system of one side.
FIGS. 18–20. Variations in Flame Cell Group 4.

EV—excretory vesicle
GR—numbered flame cell group
IN—intestine
OS—oral sucker
OV—ovary
PG—prostate gland
PH—pharynx
PL—preoral lobe
SR—seminal receptacle
SV—seminal vesicle
T_n—excretory tubules serving flame cell groups with corresponding numbers
TE—testis
VI—vitellaria
VS—ventral sucker

a tube to the posterior margin of the acetabulum, there dividing into two arms, each of which extends anteriorly, lateral to the ventral sucker, and terminates at the pharyngeal level. Remnants of the anterior commissure are observed as blind tubes extending a short distance from the expanded anterior terminations of the vesicle arms. From these terminations, a pair of long recurrent tubules extend posteriorly as far as the vitellaria where they are united by a transverse commissure which crosses the body ventral to the vitelline mass. On each side of the body, there are five short secondary tubules, each terminating with a characteristic enlargement which is joined by smaller tubules from a group of flame cells. In Figs. 16 and 17, the secondary tubules are numbered from anterior to posterior ends of the worm and their associated flame cell groups are assigned corresponding numbers to facilitate description of the excretory system.

At the level of the ventral sucker, Tubules 1 and 2 join what we have interpreted as the arms of the excretory vesicle. Tubule 1 is long and serves the anterior-most group of flame cells on its respective side of the body; the short Tubule 2 is joined by the second group of flame cells which is located at the level of the ventral sucker. Tubules 3 to 5 join the recurrent excretory tubule and serve the posterior region of the body, their corresponding flame cell groups being approximately at the levels of the testes, ovary, and vitellaria respectively.

We were unable to determine the number of flame cells in Group 3 or the exact connections of certain flame cells and capillaries in Groups 1 and 2. There is considerable variation, not only in the number of flame cells and the patterns of capillaries and small tubules in the various groups, but also in the formation of secondary connections between the recurrent tubules and the arms of the vesicle. Variations in Group 4 were studied in considerable detail since other groups were more difficult to observe in their entirety. Figs. 17 to 20 show four modifications of the pattern observed in Group 4. It is seen that the tubule connecting most of the flame cells to the expanded end of the secondary tubule may or may not form a complete loop and the number of flame cells in the group varies from eight to eleven. Since Fig. 20 represents the condition observed in a large, mature worm and has the maximum number of flame cells, it probably represents the ultimate nature of the group although the loop observed in many specimens was lacking. It is possible that such loops, like the anterior commissure, are transitory in *L. confusus*. Fig. 19 suggests that they form by fusion of one of the flame cell capillaries with the tertiary collecting tubule.

The presence of one or more tubules connecting the arms of the vesicle and the recurrent tubule as well as the direct junction of two pairs of secondary tubules with the arms make it difficult to interpret the parts of the excretory system. For this reason, our description of the vesicle as being Y-shaped with arms reaching the pharyngeal level is regarded as tentative; we are by no means certain as to where the vesicle proper ends and the collecting tubules begin. In some respects the excretory system of *L. confusus* is suggestive of the "reserve" network of certain strigeids.

SUMMARY

The life history of *Lecithaster confusus* (Odhner) has been traced experimentally. The cercaria is a small cystophorous larva developing in elongate, constricted rediae in the digestive gland of the marine snail, *Odostomia trifida*, collected from Waquoit Bay, Massachusetts, U. S. A.

Cercariae are ingested by copepods of the genus *Acartia* and develop into unencysted, active metacercariae in the hemocoele of the copepod. Adult worms were recovered from the intestine of sticklebacks, *Apeltes quadracus,* which had been fed infected copepods. The definitive host list of *L. confusus* is extended to include *A. quadracus;* also the killifish, *Fundulus heteroclitus* and *F. majalis,* all of which were found to harbor natural infections.

Development of the cercaria from the germ ball has been traced and the excretory system of the adult is described. An anterior excretory commissure is present in the metacercaria but disappears in the adult stage, indicating that *Lecithaster* is an aberrant genus of the family HEMIURIDAE. A posterior commissure is present in both the metacercaria and in the adult stages.

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RESEARCH NOTE

A NEW BACTERIUM, *CORYNEBACTERIUM LIPOPTENAE*, ASSOCIATED WITH THE LOUSE FLY, *LIPOPTENA DEPRESSA* SAY

Microscopic examinations of the crushed abdomens of specimens of the louse fly, *Lipoptena depressa* (identification confirmed by Mr. Glen M. Kohls) collected from deer in the vicinity of Hamilton, Montana, showed large numbers of a microorganism which took the red dye, as do rickettsiae, when stained by the Macchiavello method. The organisms appeared as small rods which occurred singly and in small masses. Every insect examined out of one lot of 10 harbored this microorganism which appeared to occur chiefly in the intestinal tract. No other microbial forms were encountered in these examinations.

The abdominal viscera of several specimens were inoculated into 6-day, fertile eggs. Smears from these eggs showed from moderate to few or no organisms, but transfer of triturated egg membranes to other fertile eggs resulted in abundant growth. Serial passage by means of the extraembryonic egg fluids was easily maintained through 10 transfers made at approximately 10-day intervals. The organism occurred most abundantly in the egg fluids and moderately in the yolk sac membrane.

From the egg, the organism was successfully transferred to Noguchi's leptospira medium (containing carbohydrates and minced rabbit kidney). From the latter transfers were made to glucose and sucrose agar plates, pin-point colonies resulting. The strains have since been maintained on glucose serum agar slants.

The organism is described as a new species with the following morphologic and cultural characteristics:

Corynebacterium lipoptenae n. sp.

Rods, averaging 0.8 by 4.0 microns, occurring singly and in small masses. Polar and bipolar staining in shorter forms; larger forms may be barred and slightly club-shaped. Many forms stain solidly. Non-motile. Gram-positive. No liquefaction of gelatin. Tiny, gray colonies on dextrose agar. Edges may be slightly curled, rhizoid, and ameboid. Very thin, transparent, gray, more or less filiform growth on agar slant. Rate of growth slow. Broth clear with marked sediment. No change in litmus milk. Starch not hydrolyzed. No visible growth on potato. In ordinary sugar broths, does not ferment dextrose, lactose, sucrose, maltose, mannitol, galactose, arabinose, xylose, dextrin, salicin, raffinose, trehalose, inulin, dulcitol, glycerol, inositol, adonitol, rhamnose, and mannose. Indole not formed. Nitrites not produced from nitrates. Hydrogen sulfide not produced. Aerobe (facultative). Not pathogenic for guinea pigs, rabbits, or mice.

The association of this bacterium (a symbiote?) with *L. depressa* is reported here since it may be similar to that between *Rickettsia melophagi* and the sheep "tick," *Melophagus ovinus*, which belongs to the same family (Hippoboscidae) of insects.—EDWARD A. STEINHAUS, *Associate Bacteriologist, U. S. Public Health Service. From the Rocky Mountain Laboratory, Hamilton, Montana, Division of Infectious Diseases, National Institute of Health.*

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